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ISOLATION OF NARROW SPECTRAL REGIONS BY SELECTIVE ABSORPTION

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THERE are many problems in the fields of physics, chemistry, biology, physiology, etc., which require the isolation of fairly narrow spectral regions in order that the effects produced by radiation of various wave-lengths may be determined. Obviously, the most elegant solution of the problem is the use of some type of dispersing instrument such as a monochromatic illuminator which will deliver radiation of high spectral purity. In many cases, however, it is not feasible to employ this method because of the high cost of such equipment or because of the impossibility of obtaining the required radiation intensity. For the isolation of relatively narrow spectral regions it is sometimes necessary, therefore, to use light filters made up of selectively absorbing materials. For this purpose there are many materials available, among which may be mentioned solutions, glasses, dyed gelatin, etc. Obviously, the particular method and materials used will be governed largely by the requirements of the problem.

The filters described in this paper were developed at the request of Dr. Ethel M. Luce Clausen who is investigating the action of radiation in biological experiments with the rat.* That the results might be based on an average of a large number of observations, and therefore be most reliable, this work involved the use of many of these animals. When many of these animals are to be subjected to radiation treatment, it is desirable that several be exposed at the same time. This necessitates the illumination of a relatively large area. Moreover it is desirable that the radiation intensity incident on the animals be relatively high so that

* See following paper.

the period of treatment need not be unduly prolonged. Since the treatment of a given group of animals may extend over a period of several months, it is obviously desirable that the filters be as stable as possible to avoid the necessity of constantly rechecking and making new filters.

The possibility of using a monochromatic illuminator for this purpose was considered but it appeared that the requirements of area to be illuminated and radiation intensity desired could not be met without the construction of a very special instrument which would be almost prohibitive from the standpoint of cost. A careful survey of the selectively absorbing materials available was therefore made and the filters described in the following pages were constructed.

In searching for suitable selectively absorbing materials, our attention naturally turned first to organic dyes because they are available in an almost endless variety and may be conveniently utilized in the form of dyed gelatin. The entire group of Wratten light filters was examined and the spectrophotometric absorption curves of a large number of dyes, other than those used in the manufacture of Wratten light filters, were studied. With the dyed gelatin already available it was found possible to isolate the red, green, and blue regions of the visible spectrum, excluding completely the ultra-violet. All three of these dyed gelatin filters, however, transmit freely in the infra-red; and, in fact, no dyes having appreciable absorption in the infra-red region were found.

The source of radiation used by Dr. Clausen in this work, an electric arc trimmed with white flame (therapeutic A) carbons, manufactured by the National Carbon Company, emits relatively large quantities of radiation in the infra-red region. In the study of radiation effects it is desirable to work with narrow spectral regions in order to be sure that the effects observed are actually attributable to some definite wave-length of radiation. It was necessary, therefore, to find some material for removing the infra-red radiation transmitted by the dyed gelatin filters used for the isolation of narrow bands in the visible region. It should be emphasized that in all work of this kind the visual judgment of radiation purity is of little value. Since the eye is sensitive to wave-lengths only within the region between 400 and 700 mμ, the presence of radiations of shorter or longer wave-lengths is not detectable by visual observation and in many cases workers have been grossly deceived regarding the monochromatic characteristics of the isolating filters as determined by visual observation. Several inorganic compounds were found which, when used in an aqueous solution, absorbed the infra-red region fairly well. Of these, copper sulfate ($CuSO_4 \cdot 5H_2O$) seemed most suitable from the standpoint

of stability, reproducibility, and availability. Aqueous solutions of copper sulfate of various concentrations were, therefore, used as an infra-red absorber, the concentration in any case of course being determined by the permissible absorption at other wave-lengths.

Water absorbs strongly in the infra-red and may be used for absorbing radiation of wave-lengths greater than 1000 mμ.

At the time this work was initiated the long-wave radiation limit of antirachitic radiation was very vaguely defined, it being contended by some that all radiation of wave-length greater than 305 mμ was entirely without antirachitic effect, and by others that wave-lengths as great as 350 mμ possessed a certain degree of potency. It, therefore, seemed desirable to split the region between 380 and 480 mμ into several very narrow bands. Considerable effort was expended in this direction without complete success. The filters numbered 5, 6, 7, and 8 represent the best results. A copper sulfate solution in proper concentration absorbs completely all wave-lengths less than 300 mμ, while a solution of nickel sulfate ($NiSO_4 \cdot 7H_2O$) transmits this wave-length freely but absorbs very strongly at 400 mμ. These two solutions, singly and in combination, are valuable, therefore, for controlling the absorption at various wave-lengths in the ultra-violet region (wave-lengths less than 400 mμ).

In constructing the ultra-violet filters two Corning glasses, G986A and G586, were found useful for absorbing the visible region. The former transmits freely at 300 mμ, while the latter absorbs this wave-length but transmits freely between 310 and 410 mμ. Both of these glasses absorb in the infra-red region to a certain extent. G986A is particularly useful in this respect; it has a maximum transmission at approximately 700 mμ, the absorption increasing rapidly for longer wave-lengths from this point. These two glasses are, therefore, very valuable in work of this kind. Corning glass G980 transmits freely all wave-lengths greater than 250 mμ throughout the ultra-violet, visible, and infra-red. This glass was found very useful, therefore, in the construction of a tray to hold the liquid components of the filters for the isolation of the ultra-violet bands. When it is not necessary to transmit the ultra-violet, a satisfactory tray for holding the liquid components can be made by using ordinary white optical glass. This term "white optical glass" refers to a colorless, transparent, optical glass such as is used in the manufacture of lenses, prisms, etc. The various component materials used in the construction of the filters may be summarized as follows:

Distilled water, H_2O

Aqueous solution of copper sulfate crystals, $CuSO_4$, $5H_2O$

Aqueous solution of nickel sulfate crystals, $NiSO_4$, $7H_2O$
Glass, Corning No. G986A
Glass, Corning No. G586
Glass, Corning No. G980A
Dyed gelatin, Wratten filter No. 88A
Dyed gelatin, Wratten filter No. 25
Dyed gelatin, Wratten filter No. 61
Dyed gelatin, Wratten filter No. 49.

The construction of a suitable container to hold the necessary liquid components was given careful consideration. Past experience has shown that the use of a liquid component in light filters is usually very troublesome since it is difficult to make a container which will not leak when used over long periods of time. The final solution in this case was found in the form of a shallow tray, the bottom of which is composed of six glass plates set into rectangular openings. Constructional details of this tray and drawings illustrating it have been given by Dr. Clausen.[1] The dimensions of the tray, 19½ inches long by 11½ inches wide, were adjusted to conform to the irradiation equipment already available. The glass plates forming the bottom of this tray are 5½ inches by 4⅝ inches and when mounted have a clear effective area of 5 inches by 4¼ inches. The total transmitting area of the tray is therefore approximately 120 square inches. This tray sits on top of the irradiation housing, the source of radiation being placed immediately above, and the animals to be irradiated directly under, the tray in a light-tight enclosure. This tray therefore constitutes a means of inserting the glass elements required, the liquid component being held within the tray. Gelatin components are placed beneath the tray so that they are protected from the direct radiation from the source and are thus prevented from heating and injury by exposure to the hot particles which fall from the arc during operation. These gelatin filters are mounted in a wooden frame, also arranged in the form of six rectangular sections similar to a window sash. The openings in this frame correspond to the openings in the tray. This wooden frame, carrying the gelatin film, slides into a horizontal slot just under the tray and when in position completely closes the housing so that no stray light may enter. The glass plates are held in position by means of rectangular metal frames attached to the bottom of the tray with screws. In assembling the tray a pure unvulcanized rubber gasket is first placed in contact with the metal of the tray itself. The glass plate is then laid in position and a second gasket of pure unvulcanized rubber is

[1] The Use of Isolated Radiation in Experiments with the Rat. The Effect of Infra-red Radiation on the Growth of the Rachitic Rat. *This Journal*, 1929, II, 125.

laid on the glass. The metal frame is then attached and the screws holding it in position are drawn down gradually and uniformly on all sides. These holding screws are placed close together so that the pressure may be applied uniformly all around the glass plate. As pressure is applied, the gum rubber is compressed and forms a permanent, liquid-tight joint. During use this material becomes heated to a relatively high temperature and it appears that a certain amount of vulcanization takes place, thus forming an excellent, liquid-tight joint. In all cases, except that of the filter used for isolating a band in the infra-red, some component, either liquid or glass, must be used to absorb the infra-red region. It is obvious that the filter will become rather warm because of the absorption of this long-wave radiation. As a matter of fact, the copper sulfate solution rises to a high temperature and evaporation takes place fairly rapidly. After a solution has been used for some time its volume may be appreciably reduced and hence its concentration increased. It is necessary, therefore, to add distilled water in order to maintain the desired concentration.

When making the spectrophotometric measurements on the various selectively absorbing components in work of this kind, we find it necessary to include a rather great wave-length range. We know that the source is emitting to a certain extent radiations of all wave-lengths from two hundred to several thousand millimicrons. A 2-cm. layer of water is, however, included as one of the components of all filters. This material absorbs almost completely radiations of wave-length greater than 1200 and hence it is necessary, for the purposes of this investigation, actually to make spectrophotometric measurements only in the region between 200 and 1200 mμ. Since the visible region embraces only wave-lengths from 400 mμ to 700 mμ, it is obvious that the required measurements cannot be made visually. It is necessary to use photographic methods for the ultra-violet and the shorter portion of the visible spectrum. By using plates especially sensitized to the infra-red, the photographic measurements can be extended to 900 mμ. For wave-lengths greater than this, radiometric methods must be employed.

In this work four different spectrophotometers were used:

(a) A quartz spectrograph, made by Adam Hilger, with the Hilger spectrophotometric attachment. This covers the wave-length region from 200 to 600 mμ.

(b) A spectrophotometer of the Hüfner type made also by Adam Hilger. This is a visual instrument covering the wave-length range from 400 to 700 mμ.

(c) A photographic spectrophotometer covering the wave-length range

from 600 to 900 mμ. This consists of a multiple prism instrument made by Hans Heele and used with photographic plates sensitized for the wave-length region mentioned.

(d) An infra-red spectrophotometer, made by Adam Hilger, using a thermopile as sensitive element. This was used in the region of wave-lengths greater than 800 mμ.

For the benefit of those who may not be intimately familiar with spectrophotometric methods it may be worth while to explain very briefly the principle upon which photometric measurements are made. In the first place some means must be employed for dispersing the radiation into its component parts, thus forming a spectrum in which the various wave-lengths are isolated. This dispersed radiation is then separated into two equal parts by some type of beam-splitting device. The selectively absorbing material is placed in the path of one of these beams. The two parts are then brought together and the intensity of the one which has passed through the absorbing material is measured in terms of the other. In this way the absorbing power of the filter in question is determined, wave-length by wave-length.

The absorbing power of a material may be expressed in several ways. One of the most common is to state the percentage transmission, and if I_o be used to indicate the intensity of the radiation incident upon the selectively absorbing material, and I_1 the intensity transmitted thereby, then the transmission, T, is given by the expression

$$T = I_1/I_o.$$

For many purposes it is more convenient to express the transmitting or absorbing characteristics of a material in terms of density, D, where density is defined by the equation

$$D = \log_{10} 1/T.$$

The use of density, as a means of expressing this absorbing power of material, is particularly convenient because of its logarithmic form. If it is desired to determine the resultant density obtained by superposing one absorbing material on another it is necessary only to add the density values at any particular wave-length; if the absorbing power is expressed in transmissions, the two values of transmission must be multiplied together. Moreover, density is directly proportional to concentration in the case of a solution, and also directly proportional to the thickness of the absorbing layer. All the data given in the following pages relating to these filters are given in terms of density. These values are plotted as ordinates against the wave-lengths as abscissae.

The compositions of the filters finally constructed for the isolation of the desired spectral regions are as follows:

Filter No. 1—Green

Composition:	Corning glass G980A	0.32 cm.
	Solution $NiSO_4 7H_2O$ (50%)	2.0 cm.
	Wratten gelatin filter No. 40	

Absorption characteristics, spectrophotometric curve Fig. 1, Chart 1.

It will be noted that the composition of this filter is identical with that of No. 8 with the exception of the Wratten filter No. 40. This absorbs completely the ultra-violet radiation and transmits with little disturbance the green band of the No. 8 filter. As a matter of fact, the maximum transmission in the green is somewhat less, but the decrease is relatively small.

Filter No. 2—Red

Composition:	White optical glass	0.32 cm.
	Solution $CuSO_4$, $5H_2O$ (5%)	2.0 cm.
	Wratten gelatin filter No. 25	

Absorption characteristics, spectrophotometric curve Fig. 2, Chart 1.

This filter isolates the visible red. The cut on the short wave-length side of the transmission band is due to the Wratten filter No. 25, while the cut on the long wave-length side of the absorption band is due to the copper sulfate solution. It will be noted that there is a slight residual transmission with a maximum at 1080 mμ; the minimum density, however, is relatively high, being 2.35, which corresponds to a transmission of 0.44 per cent. It is considered that this is so small as to be negligible.

Filter No. 3—Infra-red

Composition:	Corning glass G986A	0.32 cm.
	Water	2.0 cm.
	Wratten gelatin filter No. 88A	

Absorption characteristics, spectrophotometric curve Fig. 3, Chart 1.

This filter represents the best that we have been able to do in the isolation of a narrow band in the infra-red. The cut on the short wave-length side is due to the gelatin component, Wratten No. 88A, while that on the long wave-length side is due to a combination of the absorption of Corning glass G986A and the 2-cm. layer of water.

Filter No. 4—Blue

Composition:	White optical glass	0.32 cm.
	Solution $CuSO_4$, $5H_2O$ (5%)	2.0 cm.
	Wratten gelatin filter No. 49	

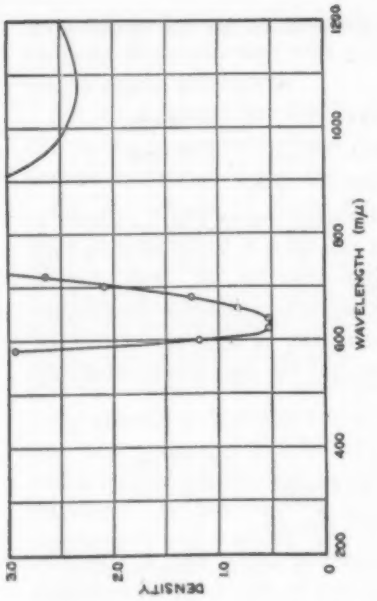

FIG. 2

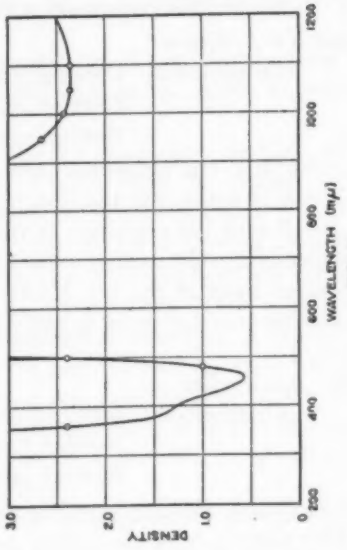

FIG. 4

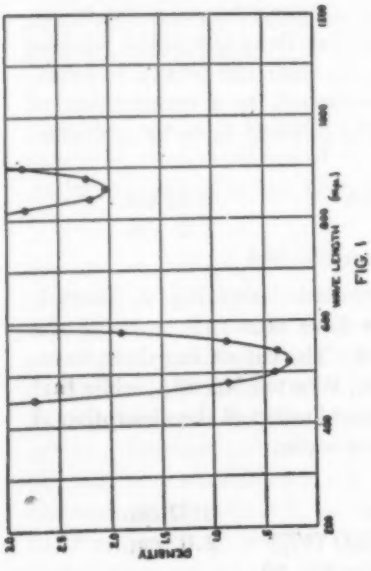

FIG. 1

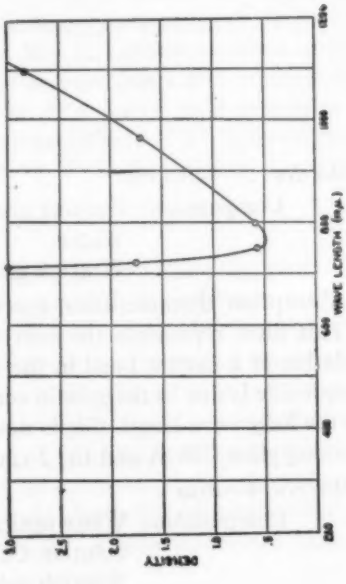

FIG. 3

CHART 1. Spectrophotometric absorption curves for filters Nos. 1, 2, 3, 4.

Absorption characteristics, spectrophotometric curve Fig. 4, Chart 1. This filter isolates the blue region of the visible spectrum, including a little of the near ultra-violet. Again there is a slight residual transmission in the infra-red which is considered negligible.

Filter No. 5—Ultra-violet

Composition:	Corning glass G986A	0.32 cm.
	Solution $NiSO_4$, $7H_2O$ (50%)	2.0 cm.
	Solution $CuSO_4$, $5H_2O$ (1%)	

Absorption characteristics, spectrophotometric curve Fig. 5, Chart 2. This filter isolates a band in the ultra-violet between the limits 270 and 355 mμ with a maximum transmission at 315 mμ. This maximum transmission is, however, relatively low, being approximately 19 per cent. This filter, therefore, although very satisfactory from its position in the spectrum, cuts down the intensity of the radiation more than is desirable. The limit on the short wave-length side of the transmission band is due to the copper sulfate solution, while the limit on the long wave-length side is set by the nickel sulfate solution. The Corning glass G986A serves to absorb the visible radiation and also, when combined with the copper sulfate solution, completely eliminates the infra-red radiation. This filter, therefore, gives a relatively homogeneous band of radiation undiluted by residuals of any other wave-length.

Filter No. 6—Ultra-violet

Composition:	Corning glass G586	0.32 cm.
	Solution $NiSO_4$,$7H_2O$ (20%)	2.0 cm.
	Solution $CuSO_4$, $5H_2O$ (5%)	

Absorption characteristics, spectrophotometric curve Fig. 6, Chart 2. This filter transmits a very narrow band having a maximum transmission at 340 mμ. It is similar to filter No. 5 but with the transmission band moved slightly in the direction of longer wave-lengths. This displacement is obtained by increasing the concentration of copper sulfate solution, thus absorbing all wave-lengths shorter than 315 mμ; and by decreasing the concentration of the nickel sulfate thus moving the cut, caused by this material, somewhat in the direction of the long wave-lengths. Although this filter is almost ideal from the standpoint of narrowness and the consequent purity of radiation, its high absorption is a serious disadvantage in that it unduly decreases the intensity factor of the transmitted radiation. Unless measures are taken to increase the intensity of radiation incident on the filter or to prolong the exposure time, it may

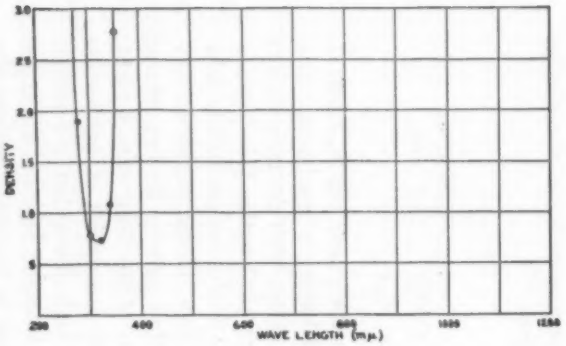

FIG. 5

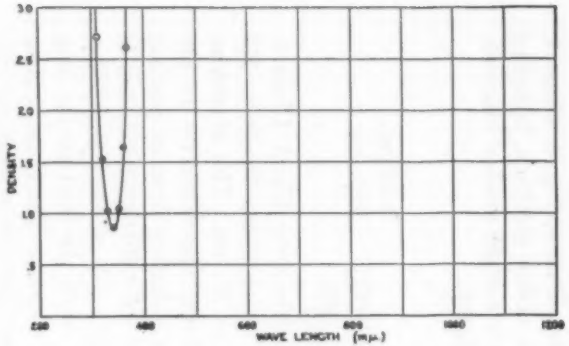

FIG. 6

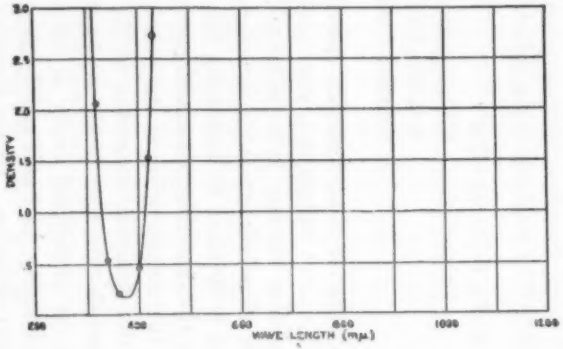

FIG. 7

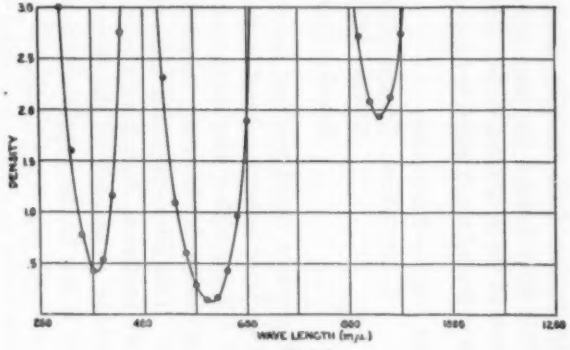

FIG. 8

CHART 2. Spectrophotometric absorption curves for filters Nos. 5, 6, 7, 8.

be difficult to determine definitely whether any apparent impotency of the radiation transmitted by this filter is due to its wave-length characteristics or to the low value of the radiation intensity transmitted.

Filter No. 7—Ultra-violet

Composition:	Corning glass G586	0.32 cm.
	Solution $CuSO_4$, $5H_2O$ (15.6%)	2.0 cm.

Absorption characteristics, spectrophotometric curve Fig. 7, Chart 2.

This filter transmits a rather broad band in the near ultra-violet. Its maximum transmission is high, being 70 per cent at 368 mμ. It completely absorbs radiations of wave-length less than 317 mμ. The cut on the short wave-length side of the band is determined by the copper sulfate component while that on the long wave-length side is determined by the Corning glass G586. Although the filter is very satisfactory from the standpoint of total transmission characteristics, the transmission band is somewhat broader than is desirable.

Filter No 8—Ultra-violet

Composition:	Corning glass G980A	0.32 cm.
	Solution $NiSO_4$, $7H_2O$ (50%)	2.0 cm.

Absorption characteristics, spectrophotometric curve Fig. 8, Chart 2.

This filter has a double transmission band, one having a maximum at 304 mμ in the ultra-violet and the other at 524 mμ in the mid-green of the visible spectrum. The residual transmission band having its maximum at 830 mμ in the infra-red may be neglected since the maximum transmission in this region is only 12 per cent. It will be noted that the absorption of this filter is due entirely to the nickel sulfate solution, since the G980A glass is completely transparent between 200 and 1200 mμ. The characteristics of the transmission band in the ultraviolet are excellent for the purposes for which these filters were designed. The maximum transmission, at 340 mμ, is 40 per cent, while the limits extend from 240 mμ to 357 mμ. Thus far no absorbing material has been found which will eliminate the transmission in the green without seriously interfering with the transmission in the ultra-violet.

In some of the preliminary experiments Dr. Clausen found that very positive results were obtained by using filter No. 8. The question at once arose whether the effect was due entirely to the radiation in the ultra-violet, or was dependent to some extent upon that in the green. Filter No. 1, since it is practically identical with No. 8 in respect to its green transmission, was therefore used in later experiments to determine de-

finitely whether or not the presence of the green radiation transmitted by filter No. 8 along with the ultra-violet had any significance.

In Table I are summarized the characteristics of the eight filters described in this paper. In the column "wave-length limits" are shown the wave-length values at which the transmission of the filter is equal to 0.1 per cent (D = 3.0). It is considered that any transmission less than 0.1 per cent may be neglected and these wave-length values represent satisfactorily the "limits" of transmission. In the column "CG" are given the wave-length values which are estimated to represent the center of gravity of the transmission band. This in most cases is very nearly equivalent to the wave-length of the maximum transmission, but in case of asymmetrical transmission bands the two values may differ appreciably. In the last column of the table, "E," are the values of total energy transmitted by the various filters.

TABLE I

SUMMARY OF CHARACTERISTICS OF FILTERS

No.	Color	Transmission Maximum	%tr.	Wave-length Limits	CG	Residuals	E
1	Green	522	55	443–590	524	805 to 903 max. at 830 Tr. = 1.2%	0.042
2	Orange red	630	30	580–725	640	910 to 1300 max. at 1080 Tr. = 0.44%	.040
3	Infra-red	770	35	720–1120	860		.122
4	Blue	455	26	360–505	435	910 to 1300 max. at 1080 Tr. = 0.44%	.030
5	Ultra-violet	315	19	270–355	316		.005
6	Ultra-violet	340	14	315–370	340		.003
7	Near Ultra-violet	368	70	317–430	376		.012
8	Ultra-violet	304	40	240–357	305		.113
	and green	524	75	416–605	525	804–903 max. at 830 Tr. = 1.2%	

The determinations of total energy transmissions were made under actual working conditions. These values were measured by means of a thermopile and sensitive galvanometer. The response of a thermopile depends upon thermo-electric forces generated by the absorbed energy

and is independent of the wave-length. Readings obtained are therefore directly proportional to total energy incident upon the thermopile. The thermopile was placed in the irradiation housing and occupied approximately the same horizontal plane as that occupied by the rats during the irradiation treatment. The light source was then operated under the same conditions as those used in the treatment of the animals. It is practically impossible to operate an arc lamp at constant intensity, since there are continual fluctuations in current which are due to several causes. It was necessary, therefore, to make several readings in order to obtain a satisfactory value. Working in this manner, we found it was impossible to obtain very good agreement between successive readings. A more elaborate arrangement was therefore made under laboratory conditions where the arc could be more precisely controlled than in practice. A set of filters, identical with those used in the irradiation work, was arranged and a second set of measurements made. The values given in Table I are the weighted mean of these two sets of observations. The absolute value of energy of course must be derived entirely from those measurements made under actual working conditions in the irradiation housing. The energy measurements made in the laboratory are somewhat more precise and reproducible and this series was given greater weight in determining the relative energy values of the various filters. The values given in Table I are in terms of calories per square centimeter per minute. It will be noted that filters Nos. 5 and 6 transmit relatively very little energy. The value for filter No. 7 also is relatively low. The energies transmitted by filters No. 3 and No. 8 are approximately equal. That transmitted by No. 1, when subtracted from that transmitted by No. 8, gives approximately the value of energy which is transmitted by the ultra-violet band of filter No. 8. A comparison of the green transmission bands in filters No. 8 and No. 1 will show that that of No. 1 is somewhat narrower and has a lower transmission maximum. It is probable, therefore, that in arriving at a value for the energy transmitted by the ultra-violet band of No. 8 a value somewhat greater than 0.042 should be used. It is estimated that the energy transmitted by the green and infra-red bands in filter No. 8 is approximately 0.050 calorie per sq. cm. per minute. This gives for the ultra-violet band of filter No. 8 a value of 0.63. This value is practically 50 per cent of that for filter No. 3.

THE USE OF ISOLATED RADIATIONS IN EXPERIMENTS WITH THE RAT

I. THE EFFECT OF INFRA-RED RADIATION ON THE GROWTH OF THE RACHITIC RAT

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Introduction

Of RECENT years the study of radiation therapy has received a great impetus. The discovery of the value of ultra-violet radiation in the prevention and cure of rickets is so well known as to need little comment. Since this discovery, the use of radiation therapy has been extended to the treatment of many diseases and exaggerated claims have been made as to its value. Very little attention has been paid to the fact that full radiations from any source of light may include a wide range of rays extending from the ultra-violet, through the visible to the infra-red regions of the spectrum.

There is a tendency, in an ever-growing literature on the subject of light, to attribute every effect observed to radiations from the ultra-violet end of the spectrum. There is also a general impression that neither visible nor infra-red radiation exerts a specific effect on the animal organism. In order to know whether this is the case, it is necessary to isolate groups of radiations and to test their effect alone and in combination with other rays.

In 1922, Hess, Pappenheimer, and Weinstock (1) and Hess and Weinstock (2) realised the importance of working with light filters. In an attempt to isolate and define the limits of anti-rachitic radiations they used a series of filters and tested the effects of radiations transmitted by them on rats fed on a standard rickets-producing diet. They found that the anti-rachitic radiations were limited to a very narrow band, "between 296–302 mμ," and that waves longer than this were without effect.

The studies reported in this paper are the result of an attempt, extending over a period of two years, to work with isolated radiations, of known

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energy value and spectral distribution, and to test these radiations on rats suffering from various dietary deficiencies.

The filters used in these experiments have been supplied to me by Dr. C. E. K. Mees to whom I am greatly indebted for a cooperation which has rendered this research possible, and also for much helpful criticism.

Method

The method employed in these experiments has involved the use of:

1. A series of filters made of materials with a selective absorption, which transmit narrow bands of radiation of known spectral distribution, whose energy value is measured against the source of light used.
2. A source of light giving an approximately continuous spectrum.
3. An irradiation apparatus made to fit the filters.
4. A photographic dark room for the housing of animals.

1. *Filters:* The filters used in these experiments have been designed by Mr. L. A. Jones of the Eastman Kodak Company. A full description of them has been given in this journal (3). For a series of filters of general usefulness see also L. A. Jones (4).

2. *Source of Light:* The source of light used has been the same throughout all the experiments, viz. a "Solarite" carbon arc lamp burning "white flame" copper coated carbons. ("Therapeutic A," of the National Carbon Company, 12 inch×13 mm.). The lamp was run on a direct current at 110 volts and 30 amperes and was enclosed in a brightly polished reflector as shown in Figure 1. The reflector was made of metal, plated inside with highly polished nickel to obtain a surface giving the maximum reflection of light. The lamp was suspended immediately above the irradiation apparatus as shown in Figure 1.

3. *Irradiation Apparatus:* Figure 1 is a diagram, drawn to scale, of the irradiation apparatus used. It consists of a strong wooden box supported on a table by three levelling screws. (Figure 1, diagram 1, gives a longitudinal, diagram 2 a transverse, view in section of the apparatus). The box is blackened inside and carries two metal ventilators above and below at the back, and one below in the front as shown in diagrams 1 and 2. The apparatus is open below and the ventilation thus obtained was found to be completely satisfactory. Inside the box are two narrow wooden projections (E and G diagram I); for holding an irradiation cage (G) used for rats, and a tray (E) used for the raying of diet. Filters A and B fit the apparatus as described below.

Filter A fits directly into the top of the wooden box. It consists of a metal tray 11 inches wide, 18 inches long and 2 inches deep, made of cast

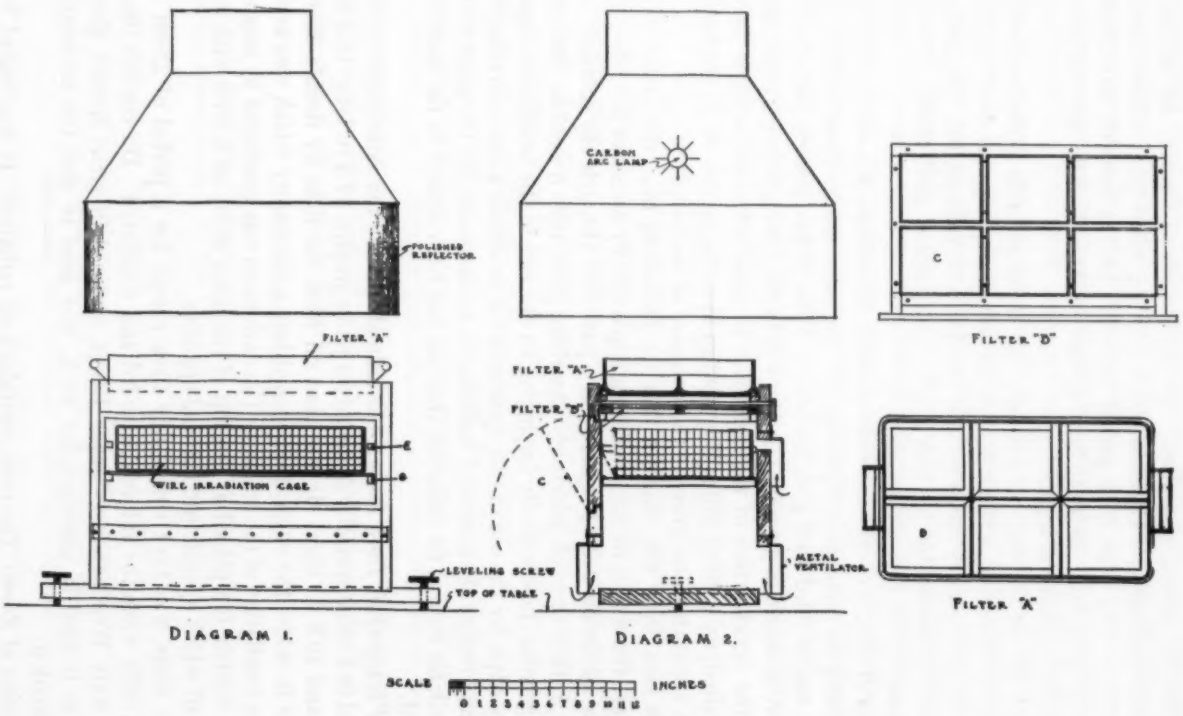

Figure 1. Irradiation apparatus.

brass heavily coated with silver. In the bottom of the tray are six rectangular openings (D) 4½ inches wide and 5½ inches long. Glass filters, protected at the edge with gasket material, can be screwed into these openings in such a way as to form a water-tight cell. This cell will hold solutions to a depth of 2 cm.

Jones (4) emphasises the necessity for using suitable gasket material in order to maintain a tight joint in these trays, during periods of irradiation. In preliminary trials we had trouble with leakage, but the gasket material recommended by him (pure unvulcanized gum rubber sheets) was found to be entirely satisfactory.

Filter B slides into the box from behind. It consists of a wooden holder containing six rectangular openings (C) the same size as those in Filter A which can be filled with gelatine filters. Filter B fits directly underneath Filter A as shown in diagram 2 and by this method the gelatine is protected from the direct action of the radiation. In cases when no gelatine filter is required, an unfilled Filter B is used so as to fill up the slit at the back of the apparatus and prevent the entrance of scattered radiation.

It is seen from this diagram that the radiations from the carbon arc lamp are thrown on to the irradiation apparatus by means of the reflector with a minimum loss of light. They pass first through the solution in Filter A, then through selectively absorbing glass, then if need be through dyed gelatine, before finally impinging on the rats in the irradiation cage. It is possible by the use of this apparatus (a) to obtain a concentration of light and reduce the scattered radiation to a minumum; (b) to ensure that the animals receive no radiation that has not been filtered in the manner desired.

4. *Photographic Dark Room:* The animals used in the experiments were housed in a room specially designed for photography, 17.4 ft. long; 16.4 ft. wide and 10.8 ft. high. It was shut off from the light by double doors each 3 ft. wide; the outer door opened into a laboratory which was used for the irradiation of the animals. The dark room was ventilated by means of an electric fan placed at the top of the inner door, on a level with an air shaft which opened into the wall opposite.

The room was kept completely dark except for a period of about 2 hours daily when the animals were fed and irradiated. During this time a 10 watt Westinghouse Mazda Lamp, enclosed in green frosted glass such as is used in photographic work, was used to give the necessary illumination.

Heating of Room: The room contained no radiators. It was heated by means of hot air pipes covered with an insulating material containing

80 per cent magnesia. The amount of heat from the pipes maintained a fairly constant temperature of 70° F in the room during the winter. In summer it rose higher, as discussed later.

The rats were kept in galvanised iron wire cages (8×10×10 inches) made with a ½ inch mesh, and had no bedding. Food and water were supplied in glass jars which were changed daily. Each animal was weighed once a week. The cages were supported on wire stands 1½ inches high with a zinc pan placed underneath to catch excreta. Faeces and urine fell through the meshes of the cage to the pan beneath, and the daily technique of keeping the animals clean simply consisted in replacing soiled by clean pans. The cages were kept on tables covered with oilcloth, which were washed daily when the pans were changed. This technique for the care of the animals was found to be both quick and efficient. Speed was necessary in view of the desirability of having the room illuminated (even dimly) for the minimum time each day.

Daily Routine of Irradiation

The carbons of the lamp were first adjusted to ensure that they would burn for the required length of time. Filter A filled with the required glass was then fixed in position, and the necessary solution poured in to a depth of 2 cm by measurement; Filter B, (filled or unfilled with gelatine) was placed in position and the level of the solution in Filter A was tested in all the cells and adjusted if necessary.

The rats were then brought out from the dark room in a closed container (6 to 9 could be rayed at one operation) and put into the wire cage. A wooden door (C diagram 2) which fitted in front of the irradiation cage was immediately closed. The lamp was then turned on for the required length of time, then shut off, and the rats were transferred back to the dark room in the closed container.

Re-measurement of Filters

Fresh solutions were not made up daily, but were kept up to the required concentration by the addition of distilled water to compensate for evaporation. About 3 liters of the solution to be used were made up at the beginning of the experiment. Enough of this to give a level of 2 cm. in Filter A (Figure 1) was used. After use each day the solution was washed back from Filter A into the stock bottle. A sample of each solution used was remeasured after daily use during an experiment lasting for 10 weeks. No change had occurred in the absorption of any of the solutions during this time. This is evidence (a) of the stability of the ingredients used in

the different solutions; (b) that it is possible with care to keep the concentration constant by the addition of distilled water, and so to avoid the necessity of making up new solutions.

Spectrophotometric measurements were made of the glass filters at the beginning and end of experiments to determine whether any change of absorption had occurred with use. It has been found that certain filters which transmit ultra-violet radiation are unstable. These filters may become opaque to short wave-length radiation after prolonged exposure to light of high intensity. We found that the intensity used in our experiments was not sufficient to alter the transmitting power of the glass filters used. Samples measured before and after use showed identical absorption curves. The re-measurement of filters after use is, however, a necessary precaution.

Care of Filters After Use

After use each day the solution in Filter A was washed out and the filter carefully dried and polished. The gelatine filter was also removed from the apparatus and kept free from dust. Care was also taken to keep the inside of the reflector well polished. These apparently trivial points are emphasised because of their extreme importance in experiments whose aim is the quantitative use of radiation.

Experimental Results in a Preliminary Experiment on the Effect of Raying Rachitic Rats through a Series of Filters Transmitting Narrow Bands of Radiation

The rachitic rat was used in these experiments because of its known susceptibility to ultra-violet radiation.

In an orientation experiment we used seven filters, full details of which are given by Jones (3) (Filters 2, 3, 4, 5, 6, 7, 8).

Three families of rats, containing 8 members in each family (from the Albino Supply Co., Philadelphia) were used in this experiment. They were divided into 8 groups, each group of three rats containing one member of each family. All the rats were fed on Steenbock's modification (5) of McCollum's 3143 diet. One group was kept all the time in complete darkness, the other seven groups were rayed daily for 10 minutes, each group of three rats receiving radiations through one of the seven filters. The experiment was continued for a period of 70 days. At the end of this time the rats were killed, and an ash analysis was made of the bones of both legs, (femur, tibia and fibula). The bones were dried for 12 hours at

110° C, extracted in hot alcohol for 48 hours, followed by 12 hours extraction in hot ether. The fat-free bones were then ashed in porcelain crucibles at red heat till all organic matter had disappeared.

Result of Experiment: The main result of this experiment is seen from Fig. 2 where the average ratios between the ash and organic residues (A/R values Chick and Roscoe (6)) in the bones of each group, are plotted against the wave-length at the centre of gravity of the band transmitted by each filter (C. G.). It is seen that the best protection, as indicated

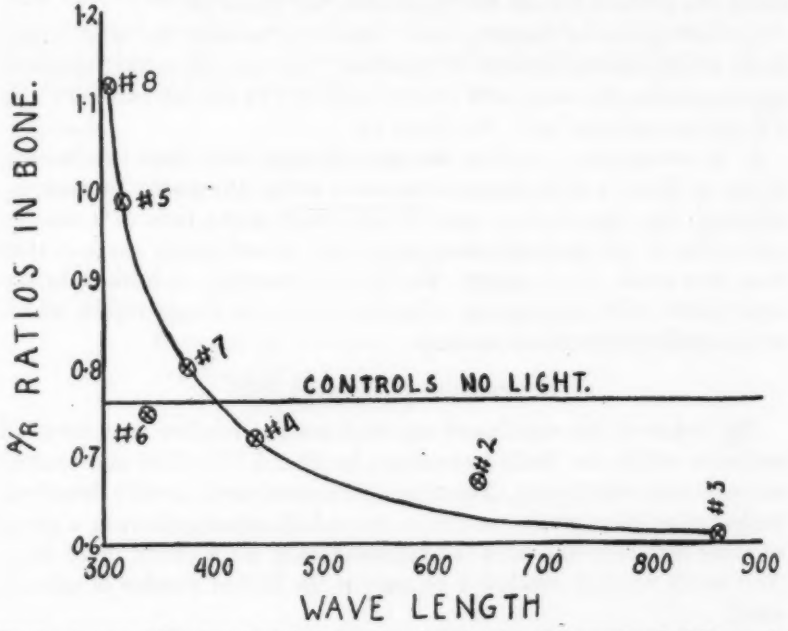

FIG. 2.—Average A/R values, 3 rats in each group, plotted against C.G. wavelength of filter used.

by the A/R ratio, was obtained with radiations transmitted by filter 8 which had a maximum transmission in the ultra-violet of 40 per cent at 304 mμ and a wave-length limit in the ultra-violet from 240–357 mμ. This filter also transmits a band of radiation in the green, which in a subsequent experiment was tested separately and found to be without effect.

This experiment illustrated some of the difficulties met with in attempts to work qualitatively and quantitatively with radiation.

1. In general, in attempting to obtain narrow spectral bands by selective

absorption methods, it is necessary to sacrifice intensity in order to obtain purity of ray. This will lead to inconclusive results such as those obtained with the use of filters 6 and 5. The results obtained with the use of both these filters can be interpreted to mean (a) That the rays transmitted were inactive; (b) That they were not transmitted in sufficient intensity to produce either a full (No. 5) or a partial (No. 6) result.

2. There is a great variability in the percentage of radiation transmitted at the wave-length of maximum transmission in each filter. The range in this series of filters is from 14–75 per cent. See Jones (3)

3. There is also of necessity great variability between the total energy value of the different groups of radiation. The two filters that transmit approximately the same total energy are 8 (0.113 gm cal/min/cm^2) and 3 (0.122 gm cal/min/cm^2). See Jones (3)

4. In attempting to analyse the data obtained from three rats in each group we found a wide range of variation within the groups themselves. Although the mean values could be expressed in the form of a smooth curve (Fig. 2), the probable error of the mean in each group was such that there was much over-lapping. We decided, therefore, to work in future experiments with large groups of animals so as to obtain results which were capable of statistical analysis.

Experiment II, Winter 1928

The object of this experiment was to determine whether near infra-red radiation within the limits transmitted by filter 3 (720–1120 mμ) exerted any effect on rachitic rats. The orientation experiment, already described, indicated an effect which resulted in lower A/R values in bone in a group of three rats receiving infra-red radiation daily for a period of 70 days. This result was not conclusive because of the limited number of animals used.

We decided to test the effect of infra-red radiation (a) given alone; (b) given in addition to ultra-violet. For this purpose we selected filters 3(infra-red) and 8 (ultra-violet). The energy value of the radiations transmitted through these two filters is approximately the same (i.e. for 3, 0.122 gm cal/min/cm^2; for 8, 0.113 gm cal/min/cm^2). Filter 8 transmits, in addition to ultra-violet, a band of radiation in the green. It has also a slight residual transmission in the infra-red. As indicated by Jones (3) Table I, the maximum transmission at 830 mμ is only 1.2 per cent with a wave-length limit of 805–903 mμ. This band is therefore very narrow; the total energy transmitted in this region must therefore be exceedingly small and, our experiments show, of negligible magnitude. We wished to find out wheth-

er the band in the green contributed to the result obtained with this filter. It was found impossible to exclude the green radiations from this filter without reducing the intensity of the ultra-violet, but the use of filter 1, Jones (3), made it possible to exclude the ultra-violet and use the green.

Full details of the radiations transmitted by these three filters are given by Jones (3) (Nos. 1, 3, 8).

Ninety rats (from the Albino Supply Co., Philadelphia) were used in this experiment; 9 litters containing 10 members in each litter. These rats were divided into five groups of 18 rats in each group, each group containing 2 members out of each family sampled by the method described below. All the rats were fed on Steenbock's modification (5) of McCollum's 3143 diet and radiated as follows:

Groups

Controls Kept in complete darkness.

1 Rayed for 10 minutes daily through filter 1.
Green radiations (443–590 mμ).

3 Rayed for 10 minutes daily through filter 3.
Infra-red radiations (720–1120 mμ).

3+8 Rayed for 10 minutes daily through filter 3.
Followed by a similar irradiation through filter 8 (ultra-violet and green)

3 720–1120 mμ
8 240–357 mμ
416–605 mμ

8 Rayed for 10 minutes daily through filter 8.
Ultra-violet and green.

240–357 mμ
416–605 mμ

Sampling of Families in the Selection of Groups

Great care was exercised in the sampling of animals for the different groups, to secure both equal representation of the different families and equal average weights of the animals in each group. Diagram I represents the method employed.

The width of the wedge indicates gradation of weight from the highest to lowest in individual members of a family, and in the mean initial weights of the families. The groups were selected as indicated on diagram. The rats were 24 days old at the beginning of the experiment and the mean initial weight per rat, in the different groups, was as follows:

	gm.
Controls	35.1±0.56
1	35.0±0.54
3	34.2±0.75
3+8	34.9±0.63
8	34.9±0.70

This method of sampling rats is emphasized because it has been found effective in reducing errors due to individual variation of the rats in the same group.

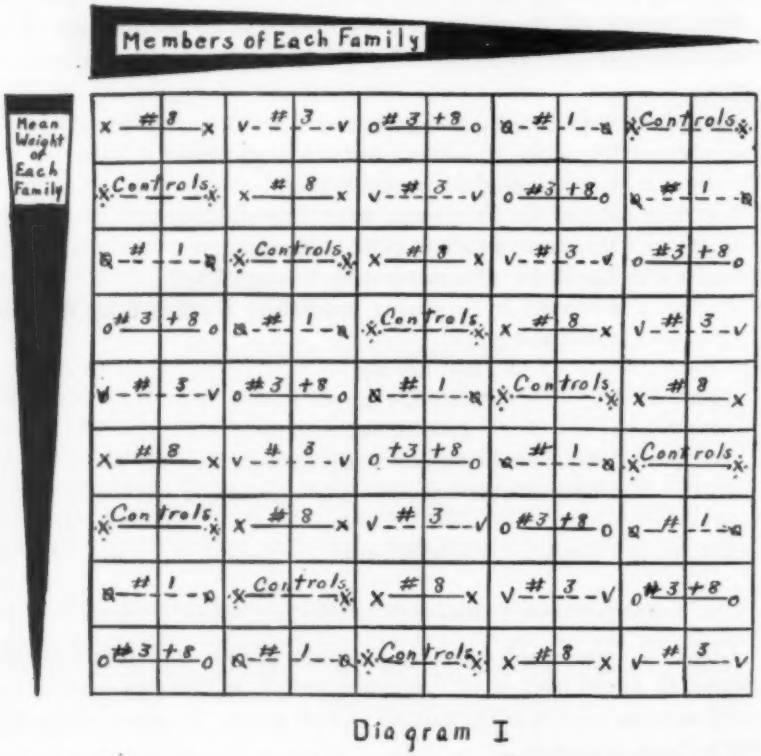

DIAGRAM I.—To illustrate method of sampling rats. Width of wedge indicates gradation of weight.

COURSE OF EXPERIMENT

The experiment was continued for 49 days, and was then terminated because the control rats and the members of group 1 were moribund with

rickets. For the first four weeks of experiment nothing very striking was observed. The different groups of rats followed the usual course of protected and unprotected rats fed on a standard rickets-producing diet, i.e. the controls group 1, and group 3 grew less well than groups 3+8, and 8, and showed no clinical evidence of receiving protection against the disease. After the 35th day, however, a striking effect on growth was

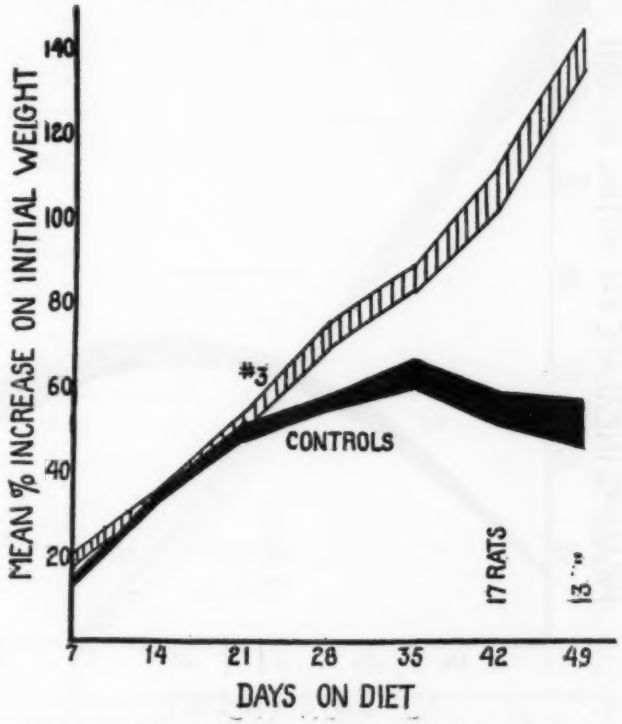

FIG. 3.—A comparison of the growth of two groups, 18 rats in each group.
Controls, no radiation.
No. 3 infra-red radiation.
Width of band—P.E. of mean.

observed in group 3 receiving infra-red radiation. At the stage in which the controls and group 1 began to lose weight, develop hind leg paralysis and all the terminal signs of extreme rickets, group 3 was in a different category. These 18 rats continued to grow, and ate about 5 grams a day more food than the controls, although clinically they showed no evidence of protection. Their chests were soft, rachitic deformities could be felt,

and there was obvious enlargement of the radioulnar epiphyses. None of them developed hind leg paralysis and they remained fairly active to the end of the experiment.

The clinical course of groups 3+8 and 8, was identical. The animals in both groups were active, showed steady growth, had firm, well developed

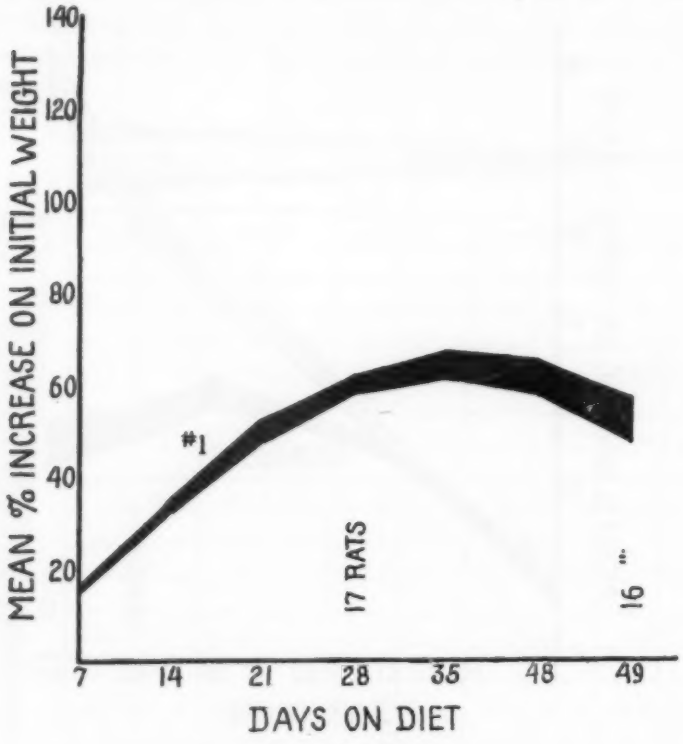

FIG. 4.—No. 1 green radiation.
Width of band—P.E. of mean.

chests and no clinical evidence of rickets. Towards the end of the experiment, group 3+8 appeared to be growing slightly better then group 8, but the difference at the time was so slight that we regarded it as being without significance. Its significance will be discussed later. Figs. 3, 4, and 5 show the growth of the different groups. In these charts the mean percentage increase on initial weight in each group is plotted against the

length of time on the diet. The probable error of the mean for each group, is indicated by the width of the band.*

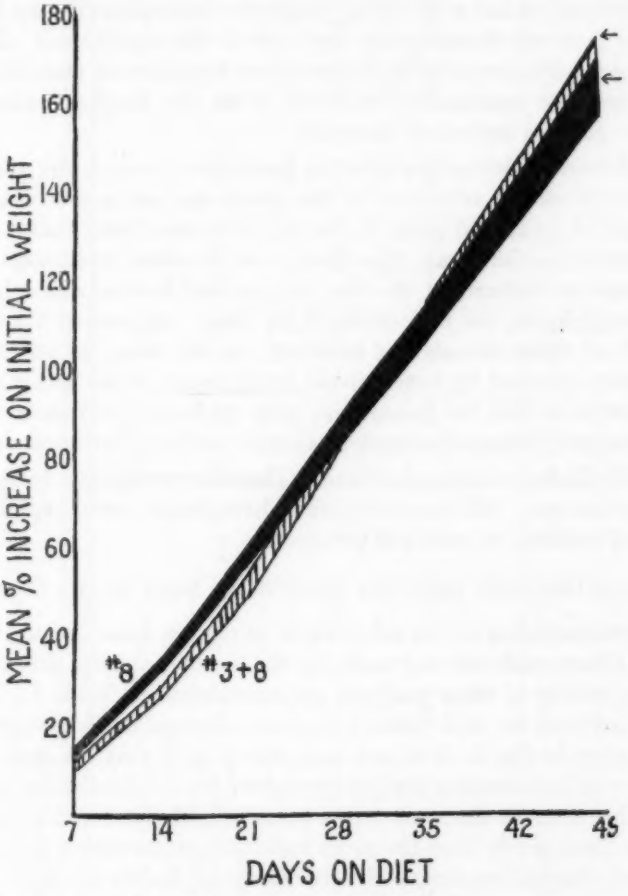

FIG. 5.—A comparison of the growth of two groups, 18 rats in each group.
No. 8 ultra-violet and green radiation.
No. 3+8 infra-red+Uv. and green.
Width of band—P.E. of mean.

*The probable error of the mean in all the experiments has been calculated from the formula:

$$\text{P. E. of Mean} = .675\sqrt{\Sigma(m-x)^2/n(n-1)}$$

m = the mean
x = any one of the observations
n = the total number of observations

Post Mortem Findings

At autopsy the rats were divided into three categories.

1. ***Extreme rickets with hind leg paralysis***—controls and group 1. These 34 rats (one out of each group died early in the experiment) all showed the same well-known clinical picture—poor nourishment, chest deformities with multiple spontaneous fractures of the ribs, beading, enlarged epiphyses, marked lordosis of the spine.

2. ***Extreme rickets without hind leg paralysis***—Group 3. By gross examination the degree of rickets in this group was just as severe as that in the control group and group 1, the only difference being that it occurred in a better nourished rat. The chests were deformed, there were multiple spontaneous fractures of the ribs, very marked beading and enlargement of the epiphyses, but no lordosis of the spine. Apparently the increased growth of these animals was sufficient, at this stage, to prevent spinal deformity followed by a mechanical involvement of the spinal cord. In a subsequent trial we found that with prolonged treatment the hind leg paralysis ultimately develops in rats receiving infra-red radiations.

3. *No Rickets*—Groups 3+8 and 8. These two groups were indistinguishable at autopsy. All the rats had firm, hard chests, normal epiphyses and showed evidence of complete protection.

Results Obtained from Ash Analysis of Bone in the Five Groups

A determination of the ash content in one leg bone (femur, tibia and fibula) from each rat was made by the method already described.

The results of these analyses are summarised in Table I. The data obtained from the A/R values have been expressed in the frequency polygons given in Fig. 6. It is seen from this (Fig. 6) that the control group, and group 1 are identical both on average values and distribution, so that the probable error of the mean is the same in both these two groups. This shows conclusively that the green radiations, transmitted through filter 1, are by themselves completely without effect. It does not show, however, whether the band of ultra-violet radiations would have been more, or less, effective without the green. So far we have been unsuccessful in isolating this band of ultra-violet radiations without reducing its intensity. It would be necessary to do this before we could say whether the band is more effective alone than mixed with the green.

This result also shows that the daily technique of handling the rats, and radiating them is completely without effect unless they are exposed to effective radiations.

TABLE I

AVERAGE VALUES OBTAINED FROM BONE ANALYSIS. 18 RATS IN EACH GROUP

Rats rayed for 10 minutes daily through filters:
1 = Green Radiations. 3 = Infra-red radiations. 3+8 = Infra-red plus ultra-violet and green. 8 = Ultra-violet and green.

GROUP	Region of Spectrum	IN BONE										
		Dry Weight of Bone Grams	% Fat		% O.R.			% Ash			% Water	A/R
			Wet Wt.	Dry Wt.	Wet Wt.	Dry Wt.	Ext. Wt.	Wet Wt.	Dry Wt.	Ext. Wt.		
Control	No Light	0.1734	4.0	10.9	24.7	68.7	77.3	7.2	20.1	22.6	64.0	0.29±0.005
1	443–590mμ	0.1859	3.9	10.0	26.4	70.1	77.8	7.5	19.9	22.1	62.2	0.29±0.005
3	720–1120mμ	0.2271	6.2	14.9	22.3	57.2	66.5	11.1	28.6	33.1	60.5	0.49±0.012
3+8	(3) 720–1120mμ (8) 240–357mμ 416–605mμ	0.3343	7.1	14.0	22.0	43.0	50.1	21.9	42.9	49.0	49.0	0.99±0.007
8	240–357mμ 16–605mμ	0.3347	7.7	14.7	21.9	41.6	58.8	22.9	43.5	51.0	47.2	1.04±0.005

The frequency polygon of group 3 is wide-spread, diphasic, and quite different in character from that of the control group and group 1. The average value for the A/R ratios in this group is higher than in the other two unprotected groups. This apparent improvement in calcification cannot be regarded as evidence of protection. The average A/R values (0.49) are well within the limits obtained for unprotected rats, and furthermore, the clinical evidence of severe rickets was unequivocal. We

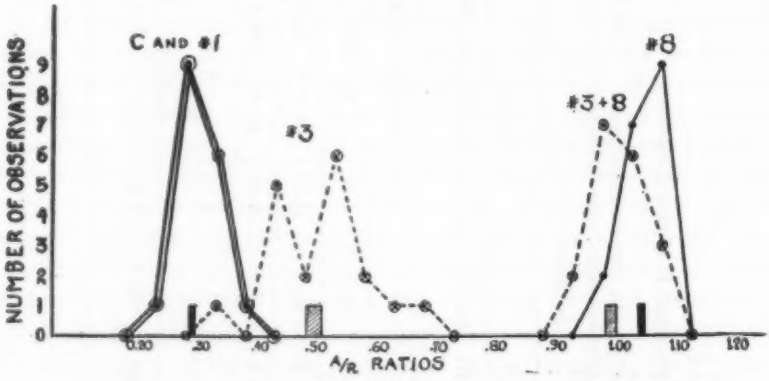

FIG. 6.—Frequency polygons of A/R ratios in bones of rats irradiated through filters.

C, controls, no radiation	17 rats	Small blocks under curves denote place of mean with P.E. of mean in each group
No. 1, green radiation	17 "	
No. 3, infra-red radiation	18 "	
No. 3+8, infra-red+ultra-violet and green	18 "	
No. 8, ultra-violet and green radiation	18 "	

attribute the higher A/R values in group 3 to an improvement in metabolic conditions following increased growth and food intake. We have shown in a subsequent experiment, prolonged for twice the time, that the improvement is only temporary.

The polygon of group 3+8 is more wide-spread than that of group 8, and the mean A/R values, including the probable error of the mean, falls below that obtained in group 8. We do not, on account of the limited number of observations, attach too much importance to the shape of these polygons, but it is interesting to note that the two groups showing the widest spread were these receiving infra-red radiation.

THE RELATIONSHIP OF THE RESULTS OF BONE ANALYSIS TO THE BODY WEIGHT OF THE RATS

The results obtained in group 3+8 as compared with group 8 were surprising. A comparison of Tables IV and V (pp. 151, 152) shows that

TABLE II

AVERAGE VALUES OBTAINED FROM BONE ANALYSIS. SUMMER 1928

GROUP	Treatment	Number of Rats	Average Number of Days on Diet	IN BONE										
				Dry Weight of Bone Grams	% O.R.			% Ash			% Fat		% Water	A/R
					Wet Wt.	Dry Wt.	Ext. Wt.	Wet Wt.	Dry Wt.	Ext. Wt.	Wet Wt.	Dry Wt.		
Control	No Light	18	52	0.1533	23.1	63.4	76.6	7.0	19.2	23.4	6.3	17.2	63.5	0.31±0.007
3	Irradiated with Infra-red. 10 mins. daily	6 Increased growth	92	0.2039	25.2	72.3	78.3	7.0	20.2	22.7	2.7	7.7	65.1	0.28±0.006
3	Irradiated with Infra-red. 10 mins. daily.	12 No increased growth	46	0.1445	21.7	62.6	76.5	6.6	19.0	23.5	6.5	18.5	65.2	0.31±0.010

group 3+8 had consistently lower A/R values in 16 out of 18 rats as compared with their litter mates in group 8. The differences are small, but consistent, and the mean A/R value including the P. E. of the mean (Fig. 6) for the group 3+8 falls well below that of group 8. This result on the analysis of bone, was the first definite indication we had that infra-red radiation given in addition to ultra-violet produced any effect, and we were at a loss to explain it.

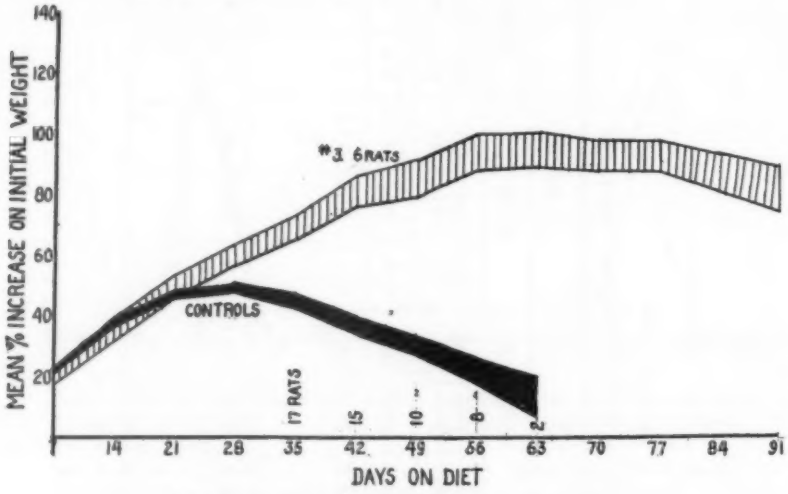

FIG. 7.—A comparison of the growth of two groups.
Controls, no radiation.
No. 3 infra-red radiation.
Width of band—P.E. of mean.

Since a lowered A/R ratio in one group must mean either an increase in the total organic residue in the bone of that group, or else a decrease in the ash, or both, we analysed the actual weights of ash and organic residue obtained from individual bone analysis of the 36 rats in these two groups. The average figures obtained were:

GROUP	O.R.	ASH
	gm.	gm.
3+8	0.1441	0.1437
8	0.1392	0.1446

These figures show that the group 3+8 had a definitely higher content of organic residue, and a slightly lower ash in bone than group 8.

We had observed a striking effect of infra-red radiation on the growth of the rats in group 3, and also a tendency to increased growth of the rats in group 3+8. We therefore analysed the data given in Chart 4 still further to find out whether the weight of ash and organic residues in the bones, bore any definite relationship to the body weight of the rats.

TABLE III

SHOWING THE RELATIONSHIP BETWEEN THE ACTUAL WEIGHT OF ORGANIC RESIDUE AND ASH IN BONE TO THE BODY WEIGHT OF RAT.

Average values obtained from 18 rats in each group.

Group	Average Body wt. Gm.	Average Organic Residue in bone. Gm.	Average Ash in bone Gm.	$\frac{\text{O.R.}}{\text{B.W.}}$	$\frac{\text{A}}{\text{B.W.}}$
Controls no light	52	0.1187 ± .002	0.0351 ± .001	0.0024 ± .00005	0.0006 ± .00002
1 Green	53	0.1297 ± .003	0.0371 ± .003	0.0025 ± .00005	0.0007 ± .00002
3 Infra-red	81	0.1287 ± .002	0.0644 ± .002	0.0016 ± .00002	0.0008 ± .00002
3+8 Infra-red+ ultra-violet and green	94	0.1441 ± .003	0.1437 ± .003	0.0015 ± .00002	0.0015 ± .00002
8 Ultra-violet and green.	91	0.1392 ± .002	0.1446 ± .003	0.0015 ± .00002	0.0016 ± .00002

The results of this analysis are given in Table III and Fig. 8. Table III gives the average weights of organic residue and ash in each group and the ratios between these and the body weights of the rats. Fig. 8 gives the frequency polygon obtained from the analysis.

It is seen from Table III that the average values for organic residue in the bones of the five groups do not vary within very wide limits, the lowest value being 0.1187 gm. in the control group, and the highest 0.1441 gm. in group 3+8. As a result of this the OR/BW ratios (Fig. 8) reflect the *growth* of the rats so that the highest values are obtained in the two groups (controls and 1) with the lowest body weight. There is a much wider range of variation in the ash content of the different groups (0.0351 gm.

in the controls–0.1446 gm. in group 8), so that the ash content of the protected rats (8 and 3+8) is roughly four times as great as that in the other groups. The A/BW ratios therefore reflect the *degree of calcification* obtained in spite of the increased body weight of groups 3, 3+8, and 8. Group 3 therefore shows an OR/BW ratio which falls within the limits of the well-grown protected groups, whilst its A/BW ratio falls within the limits of the poorly calcified unprotected groups.

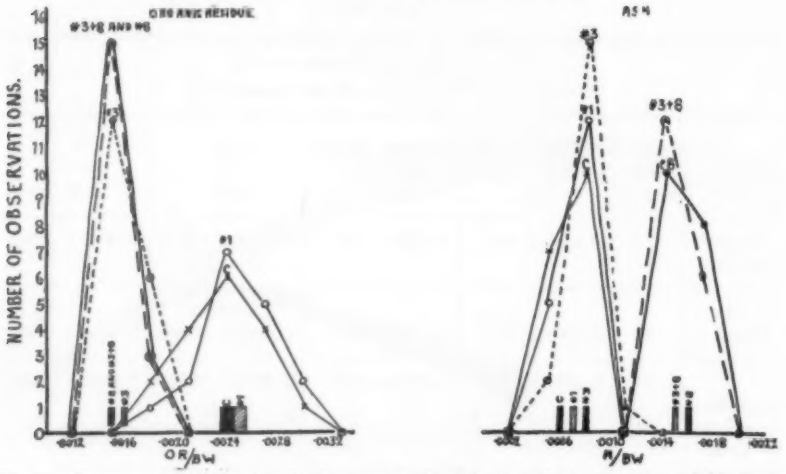

FIG. 8.—Frequency polygons of ratios between results of bone analysis and body weight of rats irradiated through filters.

C, controls. No radiation	17 rats	
No. 1, green	17 "	Blocks under curves mark
No. 3, infra-red	18 "	place of mean with P.E.
No. 3+8, infra-red+Uv. and green	18 "	of mean in each group.
No. 8, ultra-violet and green	18 "	

In Fig. 6 we have shown that the A/R ratios in the control group and group 1 are identical on mean values and distribution. The OR/BW and A/BW ratios for these two groups in Fig. 8 show slightly different distributions, but the mean values with the probable error of the mean in the two groups do not show any significant difference. The OR/BW values for group 3 are lower, and the A/BW ratios higher than these obtained in the control group and group 1, giving the higher A/R ratios shown on Chart 4.

Our main object in doing this analysis was to find out whether the lowered A/R ratios in group 3+8 as compared with group 8 could be traced to an increase in organic residue, or to a decrease in ash per unit

of body weight. Fig. 8 shows that the OR/BW ratios in groups 3+8 and 8 are identical, both on average values and distribution, so that the probable error of the mean in these two groups is the same. These two groups of rats therefore had the same amount of organic residue in bone per unit of body weight. The A/BW polygons for groups 3+8 and 8 are not quite the same. They fall within the same limits, but the shape of the polygons is different. Group 8 shows a skewed curve which may indicate a tendency for the ash values to be higher in this group than in group 3+8.

Owing to the limited number of observations, too much emphasis cannot be laid on this, but the difference is in favor of a slightly lowered ash content per unit of body weight in group 3+8.

Conclusions Experiment II—Winter 1928

(1) Eighteen rats fed on a rickets-producing diet and irradiated daily for 10 minutes with near infra-red radiation (720–1120mμ) for 49 days showed a marked acceleration of *growth* as compared with 18 litter mates kept in complete darkness.

(2) This increased growth in the rats receiving infra-red radiation was associated with slightly higher ratios between the ash and organic residue in the bones of these rats. There was no evidence of protection by clinical examination.

(3) A band of green radiations transmitted along with ultra-violet, through filter 8, had, by itself, no effect.

(4) Eighteen rats irradiated daily with both infra-red and ultra-violet radiations showed slight evidence of acceleration of growth at the end of 49 days as compared with 18 litter mates receiving ultra-violet only.

(5) This increased growth was associated with lower A/R values in bone in the group receiving infra-red in addition to ultra-violet, as compared with the group receiving ultra-violet radiation only.

(6) The two groups of rats receiving ultra-violet, with or without the addition of infra-red radiation, had the same amount of organic residue in bone per unit of body weight.

(7) The rats receiving infra-red radiation in addition to ultra-violet showed a slightly lower ash content of bone per unit of body weight, as compared with 18 litter mates receiving ultra-violet only.

(8) The main effect of a 10 minutes daily exposure to near infra-red radiation (720-1120 mμ total radiation intensity 0.122 gm. cal/min/cm^2) in both protected and unprotected rats appears to be a stimulation of growth.

Experiment III—Summer 1928

In order to confirm the result obtained in promoting growth with infra-red radiation, the winter experiment of 1928 was repeated in the summer of 1928. Thirty-six rats were used, 18 kept as controls and 18 irradiated daily for 10 minutes through filter 3. The rats were 24 days old at the beginning of experiment.

mean wt. of control group $= 35.1 \pm 0.54$
" " " group 3 $= 35.5 \pm 0.63$

These rats were sampled from litters by the method described in the preceding experiment.

Course of Experiment

This experiment was complicated by the excessive heat of summer. A large number of the rats in both groups died at a time when it was found impossible to keep the temperature of the dark room below 80°F. The highest temperature recorded was 87°F. Twelve out of the 18 rats irradiated with infra-red radiation showed no response to the treatment. Their growth corresponded with that of the controls and 9 out of the 12 were dead at the end of 49 days, the remaining 3 died at the end of 56 days.

The remaining six rats in group 3 confirmed the results already described in Experiment II. This experiment, instead of being terminated at 49 days as in experiment II, was continued with the surviving rats for 91 days. Fig. 7 shows a comparison of growth of the two groups, the controls and the six rats in group 3.

A comparison of Figs. 3 and 7 shows:

(1) That all the animals in the summer experiment grew less well than they did in the winter experiment; a result that we attribute to the excessive heat of summer conditions. The difficulties of conducting experiments with rats in the height of summer in this country are well known. It has been our experience that the vitality of the animals becomes depressed to such an extent that they may fail to respond to any form of treatment.

(2) In both experiments there was no marked divergence in growth until after the 28th day.

(3) In Fig. 7 it is seen that the six rats in group 3 continued to grow until the 56th day, whilst the controls began to lose weight after the 28th day. The six rats in group 3 maintained their weight until the 77th day and only showed a slight loss of weight by the 91st day. At the end of this time five out of the six had developed hind leg paralysis and all the signs of extreme rickets. They were then killed, since it was

evident that no protection against rickets was being obtained. If the experiment had been continued it would have been possible to keep these six animals alive for some weeks longer.

Post Mortem Findings

The control group and the 12 rats out of group 3 which failed to respond to infra-red radiation showed an extreme degree of inanition with accompanying signs of rickets and hind leg paralysis. The six rats that survived the experiment for 91 days were better nourished, but five out the six showed lordosis of the spine with accompanying hind leg paralysis, together with all the other signs of extreme rickets.

Results Obtained from Ash Analysis of Bone

These are summarised in Table II.

The average A/R values for the controls and the 12 rats in group 3 which did not respond with increased growth are identical (Table II). The level of the A/R ratios in the six rats in group 3 which did respond with increased growth fall slightly below the values obtained for the controls. This shows clearly that the increased growth in the latter group was not accompanied by any protection against the disease. It also confirms our original finding in an orientation experiment, prolonged for 70 days.

Conclusions Experiment III—Summer 1928

1. The growth-promoting effect of infra-red radiation on rachitic rats was again demonstrated in 6 out of 18 rats.
2. It is thought that the excessive heat of summer conditions of experiment interfered with the full result which was obtained in a similar experiment conducted in the winter. The temperature of the room in which the rats were kept was 10°C higher in the summer than in the winter experiment.
3. The six rats which responded to irradiation with infra-red survived the diet for at least 4 weeks longer than the controls. They ultimately developed hind leg paralysis and all the signs of extreme rickets.
4. The A/R ratios in the bones of these six rats were slightly lower than those obtained from the controls.

Discussion of Results

The literature on the subject of the biological effects of infra-red radiation is scanty and inconclusive.

Several investigators have claimed that the action of ultra-violet radiation may be modified or annulled by the simultaneous use of light of longer wave-length.

In 1922, Hess, Pappenheimer and Weinstock (2) put forward evidence that the anti-rachitic value of ultra-violet radiation was inhibited by the presence of visible and infra-red radiations. The basis of their evidence was a comparison between G86B, a Corning white glass filter, and G586A, a Corning blue glass filter, in the transmission of anti-rachitic radiations. By a comparison of spectrograms they regarded these two filters as transmitting ultra-violet radiation of the same wave-length and intensity. They found that a partial protective effect against rickets was produced in rats rayed behind the blue glass, whilst the radiations filtered through the white glass were without effect. Their argument, therefore, was that the same amount of ultra-violet radiation, whilst partially active alone, is inactive when mixed with light of longer wave-length. Azuma and Hill (7) criticised their results on the ground that they might equally well be explained in terms of unequal intensity of ultra-violet transmitted by the two filters. We know that different samples of the same glass may vary considerably in their transmission, and therefore it is impossible to say, without measuring the actual pieces used by Hess, *et al*, whether this is so. But, complete spectrophotometric measurement of samples (of the same thickness) of the two glasses made here* shows that the white glass transmits slightly more ultra-violet, and at least seven times as much total energy in the infra-red, as the blue glass. This finding would lend support to the theory of Hess.

D. T. Harris (8) in 1925 found that the addition of visible radiations nullified the stimulating effect of ultra-violet on the metabolic rate of small animals, and on the smooth muscle of the frog. He regards this phenomenon as "one of physiological antagonism, rather than physical interference."

Azuma and Hill (7) repeated the experiments of Harris and were unable to confirm them.

Sheard and Hardenbergh (9) found that the simultaneous use of ultra-violet and infra-red radiation on demodex folliculum produced lethal effects more rapidly than the consecutive application of these two types of irradiation. This would imply that the two types of radiation were more effective mixed than separate. This view is directly opposed to

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* I am indebted to Mr. E. E. Richardson of Kodak Park for his kindness in making these measurements.

that of Pèch (10) who found that the effect of ultra-violet radiation in bleaching cotton, or in the production of erythema of the skin was delayed in its action on the addition of red, or infra-red radiation.

In 1918, Bovie and Klein (11) made the extremely interesting observation of a sensitizing action of ultra-violet upon the organism. They found that the exposure of paramoecia to a sub-lethal dose of ultra-violet rendered them so sensitive to heat that they could not stand a temperature which was optimal for the controls.

A recent paper by Sheard and Higgins (12) claims that the yellow and red wave-lengths of sunlight are important for the normal growth and development of chicks. These writers regard the presence of infra-red radiation as being without effect, but as they failed to exclude it from any of their filters, no conclusions as to its value or otherwise are permissible from their data.

Most of these observations are concerned with the reciprocal action of two types of radiation; none of them postulate any independent action of infra-red, or shows that by itself it will produce any physiological effect. This field is comparatively unexplored and the results reported in this paper do not, as yet, lend themselves to any but a tentative explanation.

We have demonstrated a growth-promoting effect on rachitic rats of an isolated band of radiations in the near infra-red region of the spectrum. The effect of infra-red radiation on these animals is different from that of ultra-violet in that it confers no protection against the disease.

Given in addition to ultra-violet, infra-red radiation also produces a slight effect on the growth of rats, as compared with those receiving ultra-violet only. At the same time the rats receiving infra-red radiation show an increase in the organic constituents of bone, a slight decrease in the ash, and lowered A/R ratios, as compared with those receiving ultra-violet only. This increase in organic residue is more apparent than real since it bears a definite relationship to body weight and apparently indicates only a slight increase in growth. The ash content of the bones of the rats receiving infra-red in addition to ultra-violet radiation is slightly lower per unit of body weight, than that in the bones of the group receiving ultra-violet radiation only.

The explanation of this is, at present, a matter of conjecture. We have considered various possibilities, amongst them that infra-red radiation modifies the synthesis of Vitamin D in the skin, under the action of ultra-violet, in such a way that its full effect is not apparent. This might be brought about (a) by a direct effect of infra-red radiation upon

Vitamin D already formed, or (b) by some independent action of infra-red radiation whereby the sterols in the skin are rendered less easily activated by ultra-violet.

We found in group 3 a marked increase, as compared with the other four groups, in the total fat of the skins of the rats. This increase in fat was not accompanied by any corresponding increase in the phospholipid or unsaponifiable fractions. We did not however, find this increase in fat in the skins of the rats in group 3+8.

I am indebted to Dr. Bloor, who kindly analysed samples of the fat extracted from the skins, for permission to publish the above statement. We can draw no conclusions from our data at present.

Sonne (13) found that 1.79 gm. cal/min/cm^2 of near infra-red radiation transmitted from a carbon arc lamp could be borne apparently indefinitely on the flexer surface of the human arm without giving rise to a blister. He also found it possible to raise the temperature in the blood underneath the skin several degrees by means of visible radiations, but not with infra-red. He concluded that infra-red rays are immediately absorbed in the superficial cells of the skin which are thereby quickly heated, evoking a sensation of pain at a comparatively low energy value of radiation; and because of this more heat is absorbed from visible than infra-red radiations. His work would indicate that the effect of infra-red radiation takes place at the surface of the human skin.

With regard to the possibility of the effects we have shown being due to *heat*, we do not claim to have ruled this out and we intend to investigate it further. It seems unlikely that the small amount of total energy used 0.122 gm. cal/min/cm^2 for 10 min. per day would be sufficient to heat the animals to any great extent. It was not sufficient to raise their rectal temperatures.

We have found that rats used in our experiments are very sensitive to heat; either to increased air temperature in summer or to an overdose of heat radiation. But the effects produced on the animals in these two ways are completely different from those obtained by radiation through filter 3. When overheated, the rats show evidence of exhaustion. They become inactive, lose appetite, fail to grow, show excessive sweating which starts around the chin and neck, and if heating is continued they die. The rats irradiated through filter 3 showed none of these symptoms. They were extremely active in the irradiation cage, and showed not the slightest evidence of sweating or exhaustion. We have also shown that 12 out of 18 rats in a summer experiment, when the air temperature was 10° higher than that in the winter, failed to respond to the radiation with

TABLE IV

RESULTS OF BONE ANALYSES. GROUP 3+8

RAT		Rayed Through Filter	Region of Spectrum	IN BONE										
Serial Number	Family			Dry Weight of Bone. Gms.	% Fat		% O.R.			% Ash			% Water	A/R
					Wet Wt.	Dry Wt.	Wet Wt.	Dry Wt.	Ext. Wt.	Wet. Wt.	Dry. Wt.	Ext. Wt.		
55	A	3+8	(3) 720–1120mμ (8) 240–357mμ 416–605mμ	0.3355	10.2	19.9	21.0	40.9	51.1	20.1	39.1	48.9	48.5	0.95
56	A	"	"	0.3680	6.4	12.9	21.9	44.3	50.9	21.1	42.7	49.0	50.6	0.95
57	B	"	"	0.3507	5.0	10.1	22.2	45.0	50.1	22.2	44.9	49.9	50.6	0.99
58	B	"	"	0.3443	2.9	5.9	23.1	46.5	49.5	23.5	47.5	50.5	50.5	1.02
59	C	"	"	0.3906	7.4	14.6	22.0	43.4	50.9	21.3	41.9	49.1	49.3	0.96
60	C	"	"	0.3777	8.1	15.3	22.1	42.0	49.6	22.5	42.7	50.4	47.3	1.01
61	D	"	"	0.3322	6.3	12.9	22.3	45.3	52.0	20.5	41.7	47.9	50.7	0.92
62	D	"	"	0.3650	5.1	9.9	22.1	43.4	48.3	23.7	46.5	51.7	49.1	1.07
63	E	"	"	0.3365	9.1	17.2	21.4	40.3	48.7	22.4	42.4	51.2	47.0	1.05
64	E	"	"	0.3300	8.4	16.4	21.5	42.1	50.5	21.1	41.4	49.5	48.8	0.98
65	F	"	"	0.3065	7.1	13.9	22.8	43.1	50.1	22.0	42.9	49.7	48.6	0.98
66	F	"	"	0.2860	5.9	12.0	22.4	45.2	51.3	21.2	42.8	48.6	50.3	0.95
67	G	"	"	0.3255	7.9	15.1	21.8	41.6	49.0	22.7	43.2	51.0	47.9	1.04
68	G	"	"	0.3575	6.3	12.1	22.7	43.7	49.7	22.9	44.1	50.2	49.4	1.01
69	H	"	"	0.2670	9.6	19.4	20.8	42.0	52.1	19.1	38.6	47.8	50.4	0.92
70	H	"	"	0.3185	8.4	16.4	21.3	41.4	49.7	21.3	41.8	50.2	49.2	1.01
71	I	"	"	0.3120	7.1	13.3	22.5	42.8	49.4	22.9	43.7	50.4	47.5	1.02
72	I	"	"	0.3149	7.4	14.1	22.1	41.7	48.4	23.5	44.4	51.6	46.9	1.06
			AVERAGE	0.3343	7.1	14.0	22.0	43.0	50.1	21.9	42.9	49.9	49.0	0.99 ± 0.007

TABLE V

RESULTS OF BONE ANALYSES. GROUP 8.

RAT		Rayed Through Filter	Region of Spectrum	IN BONE										
Serial Number	Family			Dry Weight of Bone. Gms.	% Fat		% O.R.			% Ash			% Water	A/R
					Wet Wt.	Dry Wt.	Wet Wt.	Dry Wt.	Ext. Wt.	Wet Wt.	Dry Wt.	Ext. Wt.		
73	A	8	240–357mμ 416–605mμ	0.3780	6.8	13.2	22.0	42.7	49.4	22.6	43.9	50.7	48.6	1.03
74	A	"	"	0.3515	8.8	17.2	21.3	41.6	50.1	21.1	41.2	49.8	48.7	0.99
75	B	"	"	0.2782	5.4	10.7	22.0	43.5	48.4	23.5	46.0	51.5	49.0	1.07
76	B	"	"	0.3223	5.8	11.2	22.2	42.9	48.4	23.7	45.8	51.5	48.1	1.07
77	C	"	"	0.3290	5.5	11.3	21.1	42.5	49.6	21.4	43.2	50.3	50.4	1.01
78	C	"	"	0.3148	6.4	12.5	21.6	42.8	48.2	23.2	45.3	51.7	48.6	1.07
79	D	"	"	0.3212	10.7	19.7	21.2	39.1	48.7	22.3	41.1	51.2	45.6	1.05
80	D	"	"	0.4423	7.2	13.6	21.9	41.3	47.8	24.0	45.1	52.2	46.8	1.09
81	E	"	"	0.3621	9.2	16.2	22.7	40.1	47.9	24.7	43.6	52.1	43.3	1.08
82	E	"	"	0.3637	8.9	16.8	21.1	40.2	48.3	22.9	42.9	51.6	46.7	1.06
83	F	"	"	0.3185	7.6	14.2	22.7	42.3	49.4	23.2	44.7	50.2	46.4	1.02
84	F	"	"	0.3137	11.5	21.7	20.3	38.5	49.2	21.0	39.8	50.4	47.0	1.03
85	G	"	"	0.3201	7.5	14.2	22.9	43.1	50.1	22.7	42.6	49.7	46.6	0.99
86	G	"	"	0.3322	6.4	12.8	21.6	43.0	49.3	22.1	44.1	50.6	49.8	1.03
87	H	"	"	0.3130	7.5	13.9	22.1	41.1	47.7	24.2	44.9	52.2	46.3	1.09
88	H	"	"	0.2967	7.8	15.0	21.7	41.5	48.8	22.8	43.5	51.1	47.6	1.04
89	I	"	"	0.3583	8.6	15.4	23.1	40.9	48.1	24.6	43.6	51.5	43.6	1.06
90	I	"	"	0.3090	8.1	15.3	22.5	42.4	50.1	22.4	42.1	49.7	46.9	1.00
			AVERAGE	0.3347	7.7	14.7	21.9	41.6	48.8	22.9	43.5	51.0	47.2	1.04 ± 0.005

infra-red. An increase in air temperature would therefore appear to depress the vitality of the rats to such an extent that they may be rendered unable to respond to treatment.

Summary

1. A 10 minutes daily exposure to near infra-red radiation (720–1120 mμ) total energy value (0.122 gm. cal/min/cm²) from a carbon arc lamp, will stimulate growth in rachitic rats and prolong their period of survival on the rickets-producing diet.

2. The effect of infra-red radiation on the rats is different from that of ultra-violet since the animals receive no protection against the disease.

3. An increase in air temperature during summer conditions of experiment will depress the vitality of the rats, and make them less likely to respond to treatment with infra-red radiation.

4. Given in conjunction with ultra-violet, infra-red radiation also stimulates growth, to some extent, in rats fed on a standard rickets-producing diet.

In conclusion my warm thanks are due to Dr. E. A. Park for his constant support and helpful criticism throughout the course of these experiments; also to my assistant Miss Ruth Teasdale for skilled and conscientious help in tedious routine work. I should like also to thank the anonymous donor of the Gift Fund of Yale University whose generosity has defrayed the cost of this research. My thanks also are due to Dean G. H. Whipple for the hospitality of the laboratories in the Medical School of the University of Rochester, where these experiments have been carried out.

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DO BAKING POWDER RESIDUES EXERT INJURIOUS EFFECTS UPON GROWTH AND NUTRITION?

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FROM time to time attention is directed in the literature to the possibility of injurious effects resulting from the habitual use in the diet of certain types of baking powders. This is particularly true of those powders which contain tartrates or aluminum compounds as the leavening agents. Investigations in many laboratories have shown that tartrates, when administered parenterally to animals, produce a severe form of nephritis associated with profound destruction of the tubular epithelium. Several years ago studies in this laboratory (Rose, 1924) demonstrated that the subcutaneous administration to rabbits of sodium tartrate leads to enormous increases in non-protein nitrogen, urea, creatinine, sugar and cholesterol of the blood, accompanied by diminished excretion of total nitrogen in the urine. In many of the animals the ability to excrete phenolsulfonephthalein dropped to zero. Pearce and Ringer (1913) report that the characteristic renal lesions occur regardless of the mode of administration of the tartrate, *provided* the latter reaches the circulation. On the other hand, it has been clearly shown that when tartrates are given by mouth, much larger doses are required than when they are introduced parenterally (cf. Underhill, Wells and Goldschmidt, 1913; Pearce and Ringer, 1913; Salant and Smith, 1914). In human subjects, Post (1914) was unable to obtain evidence of albuminuria or cast formation after giving medicinal doses of Rochelle salts to normal individuals, nor could he demonstrate an aggravation of an existing nephritis following oral tartrate administration. Thus there appears to be no doubt as to the nephrotoxicity of tartrates injected subcutaneously; but the evidence regarding the influence of these materials when administered orally is not so clear. Furthermore, it does not follow necessarily, because a single large dose by mouth does or does not lead to renal injury, that comparable effects would result from the alimentary introduction of small amounts over long periods of time.

* Some of the earlier experiments reported herein were conducted by A. L. Rawlins.

As to the physiological action of aluminum-containing baking powders, Gies and his associates (1916) have maintained for a number of years that compounds of aluminum are absorbed from the intestine and may lead to deleterious effects. Findings adverse to the above, however, have been reported by a number of investigators. Thus the Referee Board of Consulting Scientific Experts (1914) found no detrimental influence upon metabolism following the ingestion of bread prepared with such baking powder. The experiments were conducted upon 26 healthy young men. Similar results were obtained by Schmidt and Hoagland (1919). Indeed, the latter state that aluminum is not absorbed from the intestine, but is quantitatively excreted in the feces.

Quite recently McCollum, Rask and Becker (1928) have applied the spectrographic technic to a study of the fate of administered aluminum. Although the method permits the detection of 0.5 parts per million of the metal, no evidence of the absorption of the latter from the alimentary tract was secured. The authors state that "Aluminum is not a constituent of either plant or animal matter," and that "Aluminum compounds in the diet in concentrations as high as 600 parts per million of the element aluminum exert no noticeable deleterious action on growth, reproduction, or general well being as judged by external appearance and autopsy." Similar conclusions regarding the non-toxic nature of aluminum were reached by Myers and his associates (1928). These authors observed no inhibition in growth nor other evidences of abnormality following the daily administration to rats of 2 mg. of aluminum in the form of potassium aluminum sulfate. Their results differ, however, from those of McCollum, Rask and Becker in regard to the occurrence of aluminum in biological materials. By means of a colorimeteric method developed by Myers, Mull and Morrison (1928) minute traces of the element were detected in tissues of rats and dogs, and in human autopsy material.

In view of the lack of uniformity in the data briefly outlined above, and the apparent uncertainty which still exists in the minds of many as to the possible injurious effects of baking powder components, we have undertaken a series of investigations involving the use of three types of powders containing respectively (a) a tartrate, (b) calcium acid phosphate, and (c) a mixture of calcium acid phosphate and anhydrous sodium aluminum sulfate. In the present paper we are concerned only with the alleged injurious effects, especially the influence of the baking powders upon growth and renal function. In a later communication we shall consider the distribution of aluminum in animal tissues, and attempt to demonstrate whether it is an accidental or essential dietary component.

Experimental

Young rats served as the experimental animals. These were divided into four groups, and each group was placed upon one of the diets outlined in Table I. As far as practicable, the animals of each litter were distributed among the four groups so as to make possible a comparison of the growth performance of each rat with that of its twin brothers and sisters upon the other rations. Inasmuch as the literature appeared to indicate a greater probability of nephrotoxic effects resulting from tartrates than from the other baking powder ingredients, a larger number of rats was placed upon Diet D than upon Diets A, B and C.

Table I
Composition of the Diets

	Diet A.	Diet B.	Diet C.	Diet D.
Casein, gm.	80	80.0	80	80
Whole wheat flour, gm.	760	760.0	760	760
Lard, gm.	50	50.0	50	50
Cod liver oil, gm.	30	30.0	30	30
Salt mixture, gm.	40	40.0	40	40
Yeast, gm.	20	20.0	20	20
Agar, gm.	20	20.0	20	20
Milk, cc.	530	530.0	530	530
Water, cc.	70	70.0	70	70
Calcium phosphate baking powder, gm.		64.5		
Calcium phosphate-sodium aluminum sulfate baking powder, gm.			29	
Potassium bitartrate baking powder, gm.				46

As will be observed, the diets are identical except for the baking powders which they contained. Diet A was devoid of all baking powder, and served as a control ration. The quantities of the powders employed were made to conform as nearly as possible with the directions on the containers. Inasmuch as the manufacturer's instructions are given in terms of teaspoonfuls per quart of flour, the corresponding weight proportions were determined by weighing several teaspoonfuls of each powder and several quarts of the mixed dry components of the diets. We have regarded the sum of the dry components as equivalent to the flour in ordinary bread. Inasmuch as the casein, yeast, salts, and agar, which were incorporated in our diets in order to make the food complete from the nutritive standpoint, reduced the proportion of flour in the mixture, it seemed fair to base the baking powder additions upon the weight of all ingredients exclusive of the lard and cod liver oil. On the other hand, the differences in

the proportions of the three powders employed in Diets B, C and D, are due to differences in the directions printed on the can labels.

In preparing each food the dry components, including the baking powder if present, were thoroughly mixed by hand, the lard and cod liver oil were then worked in, and finally the milk and water were added. The whole was made into a stiff dough, rolled into thin layers, cut into squares, and baked in an oven for 20 min. at a temperature of approximately 230° C. The final product was a light brown, crisp material, which was devoured greedily by the animals. From the loss in weight of the bread during baking, the food consumption of the rats, and the composition of the baking powder, it was possible to calculate the average daily intake of the leavening agents. These values will be discussed later. In addition to the bread, which was furnished *ad libitum*, each rat received 20 gm. of head lettuce at intervals of four days.

After 220 days upon the experimental rations, certain of the females upon each diet were bred with males upon the same ration. Of the young so obtained, 16 were placed at weaning upon diets identical with those of their parents with the exception that the quantities of baking powders per unit of mixed foods were doubled. Thus the second generation animals were subjected to the influences of the powders from the moment of conception until the end of the experiments. At the termination of the feeding periods the animals of both generations were anesthetized, killed by bleeding, and the blood samples subjected to analysis for non-protein nitrogen according to the procedure of Folin and Wu (1919). The kidneys were removed, weighed, and preserved for histological examination.

Seventy-two rats were employed in the investigation. In Tables II and III are shown for each animal the duration of the feeding period, the average daily intake of food and leavening agent, the final weights of the kidneys, and the non-protein nitrogen of the blood. As will be observed, the figures representing average daily food consumption of the animals upon the four diets are quite uniform for each sex. The values are all rather high, but this is to be accounted for by the fact that the food had a relatively low fat content and contained considerable moisture. A comparison of the weights of the kidneys of the animals upon the baking powder diets with those upon the control ration indicates that the leavening agents induced no hypertrophy. Indeed, the largest kidneys for each sex were found among the control animals. Rat 767 ♂ showed slightly enlarged kidneys, but the non-protein nitrogen of the blood was within the normal range for the species (45 to 50 mgm. per 100 cc). Rat 744 ♀ showed moderate hypertrophy with a very marked increase in the non-

TABLE II

FIRST GENERATION OF RATS

Rat no. and sex.	Diet no.	Duration of experiment	Average daily food consumption	Average daily intake of			Final weight of kidneys		N.P.N. of blood
				P_2O_5	Al	$C_4H_6O_5$	Right	Left	
		days	gm.	mgm.	mgm.	mgm.	gm.	gm.	mgm. per 100 cc.
756♂	A	250	15.9				1.0	1.1	45.1
763♂	A	250	16.7				1.1	1.2	44.4
767♂	A	245	16.1				1.3	1.4	47.6
		Average	16.2				1.1	1.2	45.7
728♀	A	292	12.2				0.5	0.7	45.0
733♀	A	297	12.5				0.7	0.7	44.0
744♀	A	297	13.0				1.2	1.2	101.4
749♀	A	304	13.9				0.8	0.8	44.4
774♀	A	304	12.8				0.8	0.8	52.2
781♀	A	292	14.5				0.8	0.8	47.4
		Average	13.2				0.7	0.8	46.6
738♂	B	246	15.0	182			1.0	1.0	46.4
739♂	B	250	15.3	186			1.1	1.1	46.6
753♂	B	297	16.2	197			1.1	1.1	43.4
764♂	B	288	17.3	210			1.1	1.1	45.9
772♂	B	248	14.8	180			1.1	1.2	45.8
777♂	B	248	13.5	164			0.9	0.9	45.7
		Average	15.4	187			1.1	1.1	45.6
734♀	B	299	12.8	156			0.7	0.7	44.3
735♀	B	299	12.1	147			0.7	0.7	43.3
765♀	B	307	13.8	168			0.9	0.9	46.3
		Average	12.9	157			0.7	0.7	44.6
741♂	C	250	15.0	38	10.3		1.0	1.0	45.9
754♂	C	285	14.5	36	10.0		1.0	1.0	44.7
758♂	C	250	15.5	39	10.7		1.1	1.1	43.0
779♂	C	285	15.4	38	10.6		1.1	1.2	45.1
		Average	15.1	38	10.4		1.1	1.1	44.7

TABLE II (Continued). FIRST GENERATION OF RATS

Rat no. and sex.	Diet no.	Duration of experiment	Average daily food consumption	Average daily intake of			Final weight of kidneys		N.P.N. of blood
				P_2O_5	Al	$C_4H_6O_6$	Right	Left	
		days	gm.	mgm.	mgm.	mgm.	gm.	gm.	mgm. per 100 cc.
727♀	C	292	13.0	33	8.9		0.7	0.8	45.4
755♀	C	297	14.7	37	10.1		0.8	0.8	45.0
769♀	C	304	13.6	34	9.4		0.8	0.9	53.3
771♀	C	307	14.5	36	10.0		0.8	0.8	43.7
773♀	C	303	13.7	34	9.4		0.7	0.8	44.8
		Average	13.9	35	9.6		0.8	0.8	46.4
730♂	D	247	14.7			214	1.0	1.0	47.0
731♂	D	252	15.3			222	1.0	1.1	43.5
742♂	D	285	15.4			224	1.1	1.0	44.0
746♂	D	285	15.4			224	1.0	1.0	42.6
747♂	D	248	14.9			217	1.0	1.1	45.7
748♂	D	285	14.7			214	1.1	1.1	42.8
750♂	D	252	15.5			225	1.1	1.1	43.8
752♂	D	253	16.1			234	1.0	1.1	43.6
757♂	D	285	14.8			215	1.1	1.2	46.1
759♂	D	288	15.9			231	1.1	1.2	44.3
760♂	D	288	15.6			227	1.1	1.2	45.1
775♂	D	288	14.8			215	1.1	1.1	45.7
778♂	D	288	14.5			211	1.0	1.1	45.3
783♂	D	288	14.0			203	1.0	1.0	45.5
784♂	D	248	15.9			231	1.1	1.2	43.2
786♂	D	288	16.4			238	1.1	1.1	45.4
		Average	15.2			222	1.1	1.1	44.6
729♀	D	297	12.7			185	0.7	0.7	43.4
736♀	D	297	12.6			183	0.8	0.8	45.5
743♀	D	295	12.7			185	0.7	0.7	44.4
745♀	D	295	12.4			180	0.7	0.7	44.3
751♀	D	301	14.0			204	0.9	0.9	44.6
761♀	D	299	13.0			189	0.7	0.7	44.0
762♀	D	299	13.0			189	0.8	0.8	43.4
766♀	D	292	14.6			212	0.8	0.9	45.4
768♀	D	307	14.5			211	0.9	0.9	51.2
770♀	D	301	13.7			199	0.9	0.9	47.8
776♀	D	303	12.5			182	0.7	0.7	44.2
780♀	D	299	13.6			198	0.8	0.8	42.9
782♀	D	295	14.0			204	0.7	0.8	42.9
		Average	13.3			194	0.8	0.8	44.9

protein nitrogen of the blood. Kidneys of 50 of the rats of both sexes selected at random from the four groups of the first generation, but including all of the second generation animals, were subjected to microscopic examination by Professor R. H. Jaffe of the Department of Pathology, College of Medicine, University of Illinois. Without any information as to the nature of the investigations, Professor Jaffe reported that with the exception of Rat 744, which evidently developed spontaneous nephritis, no differences were to be observed between the kidneys of the rats which received the baking powder diets and those upon the control ration. It is apparent, therefore, that evidence of deleterious effects was not revealed either by the chemical analysis of the blood or by the histological examinations of the kidneys.

TABLE III
SECOND GENERATION OF RATS

Rat no. and sex	Diet no.	Duration of experiment	Average daily food consumption	Average daily ntake of			Final weight of kidneys		N.P.N. of blood
				P_2O_5	Al	$C_4H_6O_6$	Right	Left	
		days	gm.	mgm.	mgm.	mgm.	gm.	gm.	mgm. per 100 cc.
999♂	2B	72	17.1	416			1.0	1.0	56.6
1001♂	2B	76	15.8	384			0.9	1.0	44.1
1000♀	2B	72	14.4	350			0.9	0.9	50.8
1002♀	2B	76	14.1	343			0.8	0.8	50.1
		Average	15.4	373			0.9	0.9	50.4
1005♂	2C	56	15.5	78	21.3		1.0	1.0	40.7
1006♂	2C	56	15.7	79	21.6		0.9	0.9	43.2
1003♀	2C	56	14.0	70	19.2		0.7	0.7	41.9
1004♀	2C	56	13.9	70	19.1		0.8	0.8	44.3
		Average	14.8	74	20.3		0.9	0.9	42.5
1008♂	2D	52	14.9			433	0.8	0.8	44.3
1011♂	2D	52	14.7			427	0.8	0.9	42.6
1012♂	2D	52	14.1			410	0.7	0.8	44.3
1014♂	2D	52	14.1			410	0.9	0.9	43.2
1009♀	2D	52	12.9			375	0.6	0.6	45.1
1010♀	2D	52	12.9			375	0.7	0.8	43.5
1013♀	2D	52	11.4			332	0.6	0.6	43.0
1015♀	2D	52	12.4			361	0.6	0.6	44.9
		Average	13.4			390	0.7	0.8	43.9

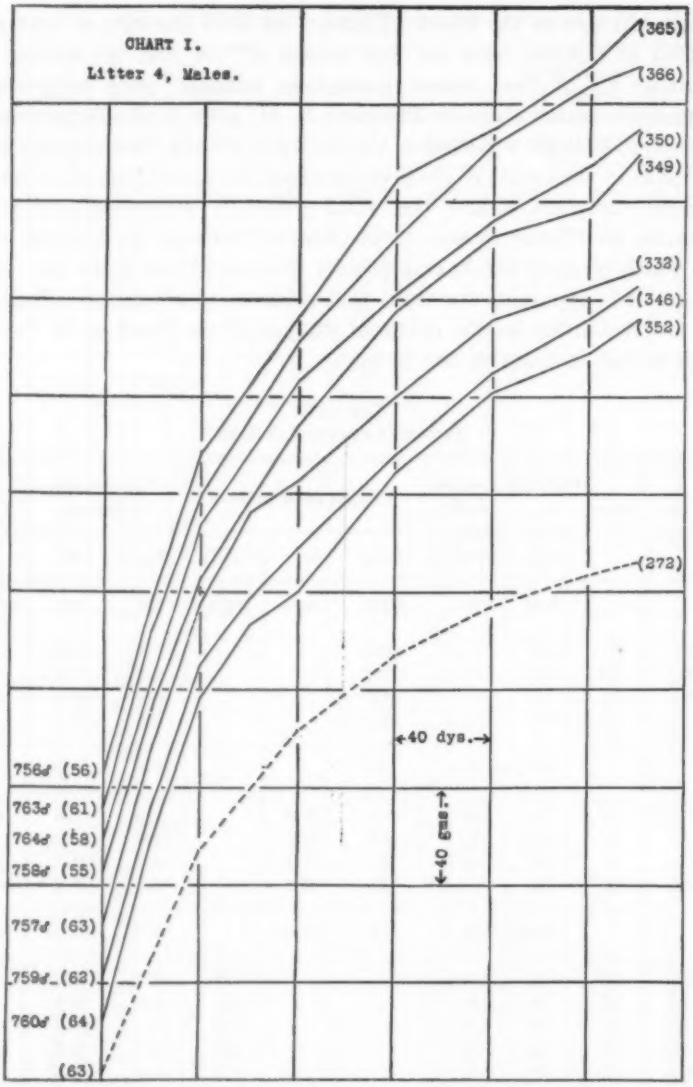


CHART I.

The numbers in parentheses denote the initial and final weights of the rats. The broken line curves show the so-called "normal" growth rate (Donaldson).

Rats 756, 763: Diet A, devoid of baking powder.
Rat 764: Diet B, phosphate powder.
Rat 758: Diet C, sodium aluminum sulfate powder.
Rats 757, 759, 760: Diet D, tartrate powder.

It has seemed unnecessary, in view of the uniformity of the data, to reproduce the growth curves of all of the first generation animals. In Charts I to III inclusive are presented the individual curves of 20 rats. These were selected because they belonged to litters the members of which were widely distributed upon the four diets. Inasmuch as many of the

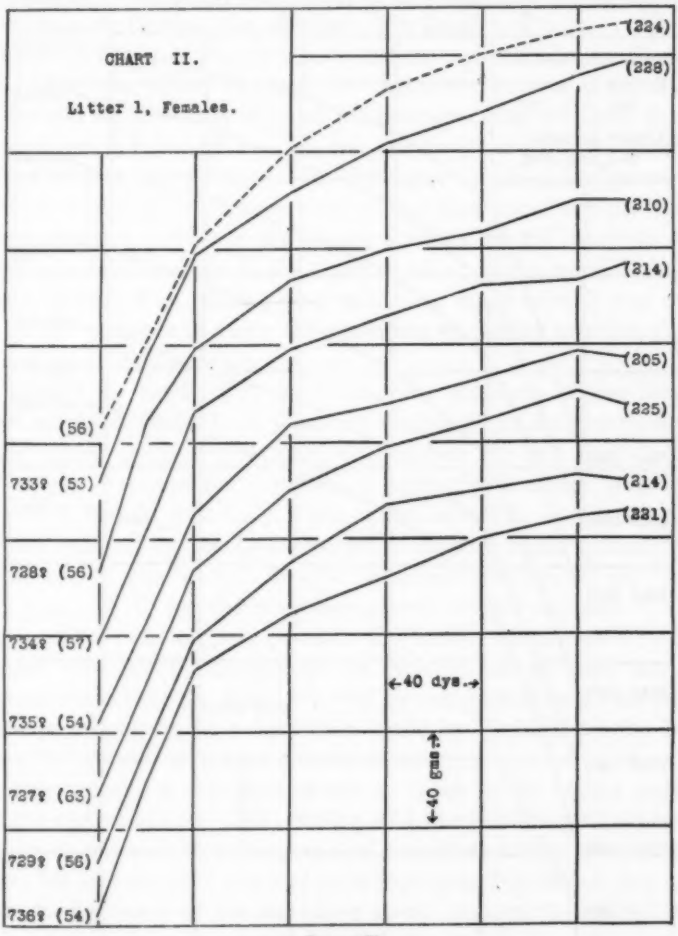


CHART II

Rats 733,728: Diet A, devoid of baking powder.
Rats 734,735: Diet B, phosphate powder.
Rat 727: Diet C, sodium aluminum sulfate powder.
Rats 729, 736: Diet Diet D, tartrate powder.

rats were bred after 220 days, the curves are shown for this period only. In Charts IV and V are presented composite curves for *all* of the first generation males and females respectively. In the latter charts the lines connect points representing the average weights at the beginning of the experiments and at the expiration of 220 days. Thus the average rate of growth of each group is indicated by the slope of the curve. This method of

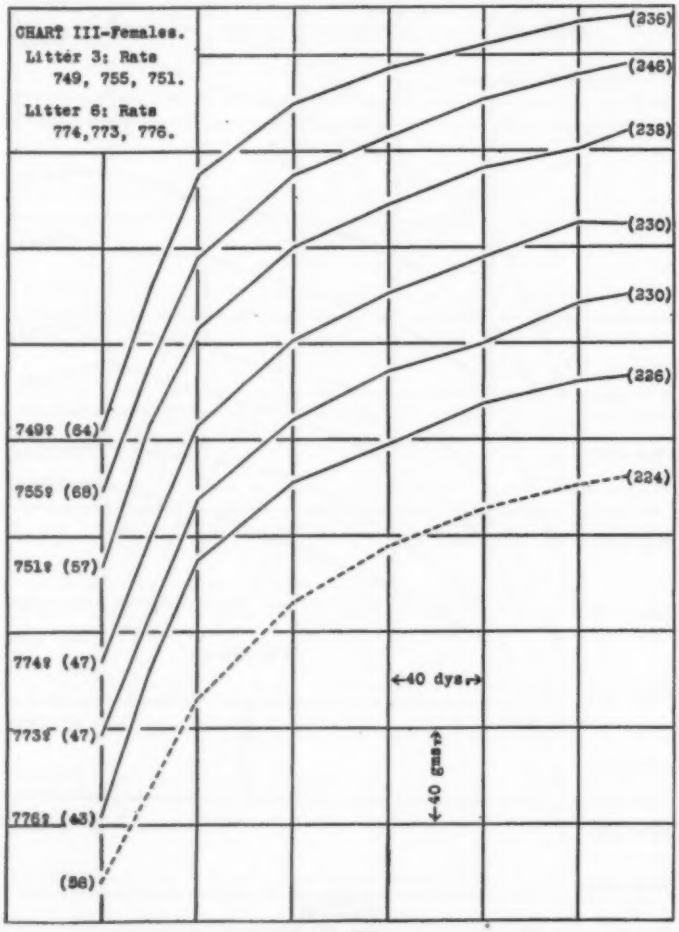


CHART III

Rats 749, 774: Diet A, devoid of baking powder.
Rats 755, 773: Diet C, sodium aluminum sulfate powder.
Rats 751, 776: Diet D, tartrate powder.

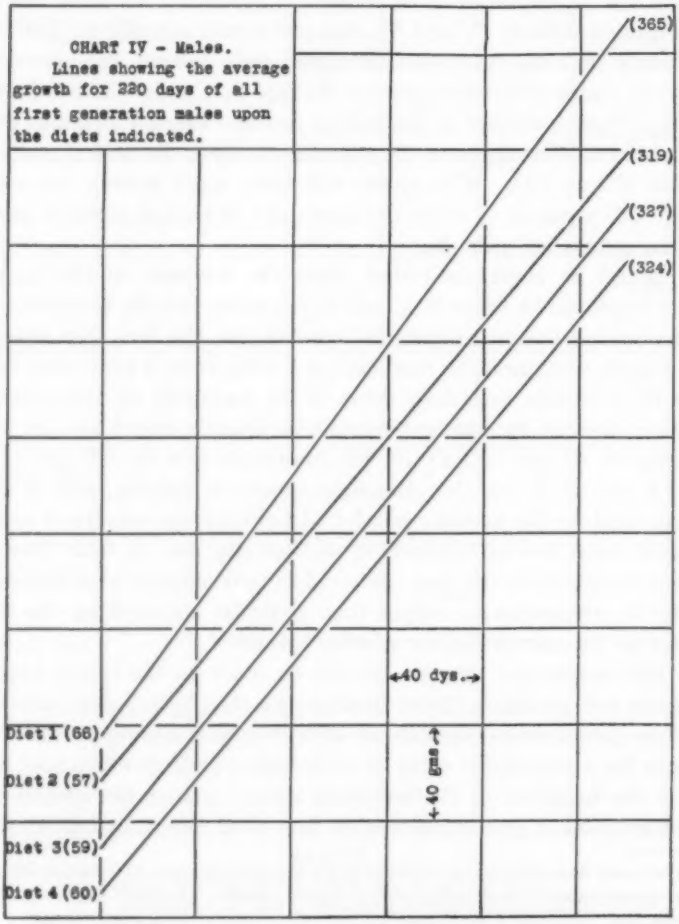
plotting appeared to afford the best opportunity of bringing out slight variations in the rates of increase in body weight. Both the individual and composite curves show quite clearly that the presence of the baking powders in the foods exerted no detectable influence upon the growth rate. All of the males (Chart I), and the females of Litters 3 and 6 (Chart III) grew more rapidly than the so-called "normal" of Donaldson (1924) for animals of the same size and sex. The females of Litter 1 (Chart II) increased in weight at almost exactly the rate indicated by the Donaldson data. The rats which received the phosphate powder (Diet B) grew slightly less rapidly than those of the corresponding sex upon the other three rations (Charts IV and V), but one would scarcely be justified in concluding that the differences are significant. Indeed, the growth data appear to afford additional proof of the fact that under the conditions of our experiments, neither of the baking powders exerted injurious effects. Similar conclusions apply to the experiments upon the second generation animals (Chart VI). With these, still more rapid growth was secured despite the presence of twice the quantities of baking powders given to the first generation animals.

It should be emphasized that while the amounts of the leavening agents employed in Diets B, C and D conformed to the directions given on the baking powder containers, nevertheless the fact that the entire food supply contained the powders led indirectly to a very large intake. Thus for a 70 kilo man daily doses of the leavening agents comparable to those ingested by our first generation animals would amount to approximately 42 gm. of P_2O_5 for the phosphate powder, 8.9 gm. of P_2O_5 and 2.5 gm. of Al[1] for the phosphate-aluminum powder, and 30 gm. of tartaric acid for the tartrate powder. Obviously, the results of such calculations must not be interpreted too literally, but at least they serve to demonstrate that the doses received by our animals were enormously greater in proportion to weight than could be obtained by the human subject in the use of baking powder breads.

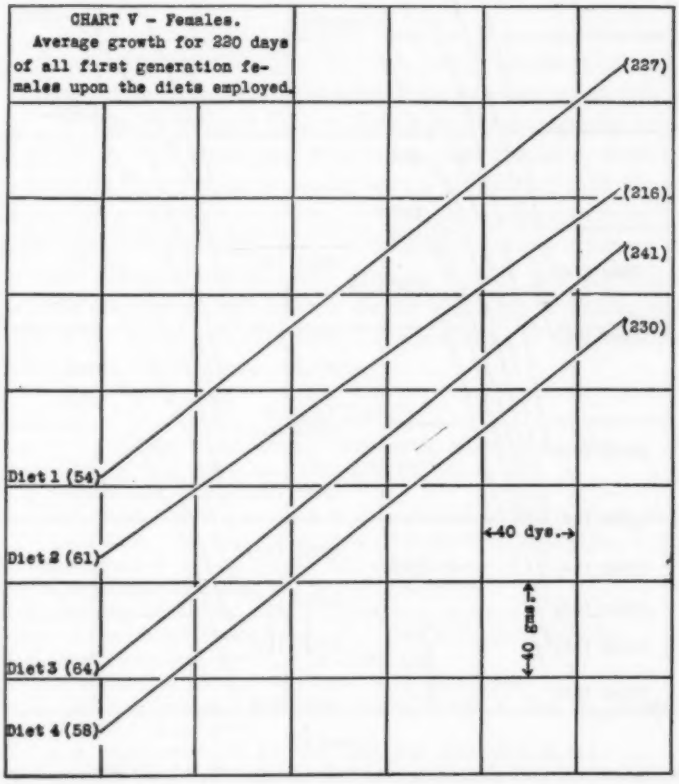
In this connection reference should be made to the recent papers of Schaeffer and associates (1928) dealing with the physiological influence of a sodium aluminum sulfate-calcium acid phosphate baking powder. These authors list a formidable array of pathological findings which they attribute to the ingestion of the leavening agent. Among the alleged effects are retardation of gastric evacuation, intestinal ulceration, diarrhoea, in-

[1] The largest dose of aluminum employed by the Referee Board was 1000 mg. per day, equivalent to approximately 10 level teaspoonfuls of baking powder. The Board points out that such an intake could occur only if one subsisted almost entirely upon baking powder bread.

hibition in growth and atrophy of the female genital organs. As subjects for the experiments the authors employed chickens, dogs, rats and mice. They state that the diets were toxic for rats only when the latter had initial weights less than 90 gms. Heavier rats were resistant to the baking powder residues. It should be pointed out that all of our animals weighed less than 90 gm., and most of them less than 65 gm. when placed upon the baking powder diets. Probably the differences in findings in the two series of experiments are to be accounted for in part by the excessive amounts of baking powder employed by Schaeffer. Thus Diet A_2 (Schaeffer, *et al.*,



1928, pp. 53 and 60) which was used for the rat growth studies, was prepared by mixing 207 gm. of baking powder with 1385 gm. of the other dry materials. Such a mixture scarcely would be expected to promote normal growth. Possibly even a *necessary* inorganic dietary component like sodium chloride might inhibit growth if included in the ration at a 15 per cent level. However, despite the excessive proportions of baking powder in Schaeffer's diet, the rats receiving it succeeded in gaining



although at somewhat slower rates than the control animals. Under the circumstances the results would appear to emphasize the *non-toxic* nature of the leavening agent. It is to be noted also that in Schaeffer's control diet (Diet L_2) bone meal replaced the baking powder of the experimental ration. The better growth which resulted suggests the possibility that the bone meal may have exerted a supplementing action. In any event the

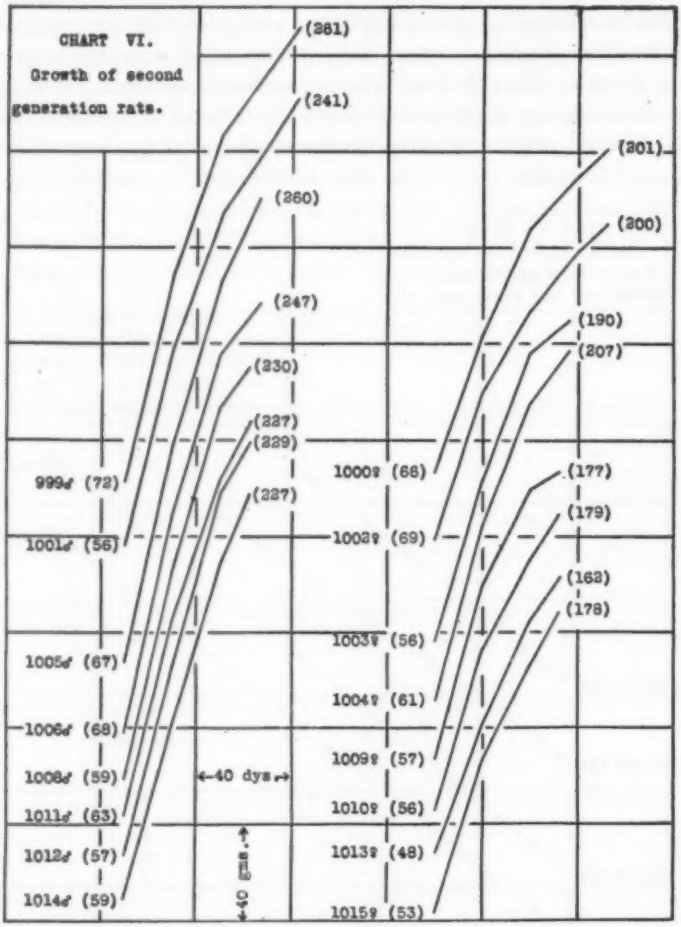


CHART VI

These animals received diets (designated in Table III as 2B, 2C, and 2D) containing double doses of the baking powders.

Rats 999, 1001, 1000, 1002: Diet 2B, phosphate baking powder.
Rats 1005, 1006, 1003, 1004: Diet 2C, sodium aluminum sulfate powder.
Rats 1008, 1011, 1012, 1014, 1009, 1010, 1013, 1015: Diet 2D, tartrate powder.

use of the meal introduced a variable other than the omission of the aluminum salt.[2] In not a single one of our experiments was there evidence of any of the abnormalities reported by Schaeffer. Our rats remained vigorous throughout the experiment, and at no time showed signs of alimentary disturbances. Nor were pathological alterations revealed at autopsy. We are convinced, therefore, that under the conditions employed by us the baking powders exerted no detectable deleterious action.

Summary

Growth experiments extending through two generations of rats have been conducted to determine whether baking powders exert deleterious effects. The results indicate that in the proportions ordinarily employed in the preparation of bread, and with twice these amounts, three typical baking powders in which the leavening agents are calcium acid phosphate, a tartrate, and a mixture of calcium acid phosphate and anhydrous sodium aluminum sulfate respectively, exert no detectable toxic or injurious action. The animals receiving the powders grew as rapidly and reproduced as satisfactorily as control rats upon a similar diet without baking powder. Analyses of the blood and histological examinations of the kidneys likewise failed to reveal evidence of injury.

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[2] Since writing the above McCollum, Rask and Becker (*Bull. Soc. Sci. d'Hyg. Alim.*, 1929, xvii, 65) have published a criticism of the paper of Schaeffer and associates to which the latter (*Ibid.* 1929, xvii, 74) have replied.

ROLLED OATS AND BRAN IN A SCURVY-PRODUCING DIET AND THE NEGATIVE CONTROL TEST

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THE early investigators of experimental scurvy used simple diets containing raw materials, whereas later investigators, in an attempt to make their diets conform to the prevailing views of nutrition, modified their dietary standards with the consequent introduction of laborious, if not actually inadvisable, procedures. Owing to the necessity for the preparation of large quantities of a scurvy-producing diet, we have investigated this problem with the object of introducing a labor saving and tested standard procedure.

This paper relates largely to the use of a diet containing rolled oats and bran with butter fat and salts. This subject was studied, first, because it appeared that oats or bran or both had been practically universally used ever since the beginning of scurvy investigations; second, because these materials seemed to be of much better feeding quality than is usually ascribed to them; and third, because they are readily obtained and make up into a diet which is very easy to handle.

Although the principles underlying the necessity of a diet free from vitamin C but adequate in other respects are known to all, it should be noted that no one has yet shown any diet to be free from vitamin C. Moreover, little or no attention has been given to the quality of subject animals, with the result that the negative control tests, as used at present, could be presumed to throw light on the quality of animals rather than on the quality of the diet, but certainly not on both simultaneously. It is obvious, therefore, that until it is established how long certain standard animals should live on a diet proven to be free from vitamin C, investigators will continue to reason in circles.

Osborne and Mendel (1) showed that 10 per cent whole oat protein in

* Contribution from the Utensil Fellowship.

the diet produced normal growth in rats, whereas the effect of 8 per cent was questionable. Smith and Hendricks (2) showed that rats grew at about one-third the normal rate over a course of 14 weeks when fed a diet containing 92.5 per cent of rolled oats, and concluded that the deficiency was due to a lack of the anti-pellagric fraction of the multiple vitamin B. Rolled oats contain about 16 per cent crude protein, very little material except the hull being removed in milling.

Osborne and Mendel (3) fed rats on bran at a 9 per cent crude protein level. Only one of four rats ate enough to grow well. Twenty per cent of the ingested nitrogen appeared in the feces instead of the more usual 10 per cent. At a 10 per cent crude protein level, growth of rats was exceedingly good for Murphy and Jones (4) during the first 100 days. The slight relative deficiency appearing later was made good with 5 per cent of patent flour. Jones and Gersdorf (5) found that bran is liberally supplied with all of the so-called essential amino acids. Bell and Mendel (6) found that 20 per cent of bran in the diet contained sufficient vitamin B complex to produce normal growth in mice, whereas 10 per cent was insufficient. Bran contains about the same amount of crude protein as rolled oats.

Hess (7) states that wheat germ possesses some antiscorbutic value. Jones and Gersdorf (5) found no evidence of germ in their sample of bran but estimated, on the basis of starch content, a possible 0.5 per cent.

Chick and Delf (8) used a diet of oats and bran and obtained good growth in guinea pigs fed with 30 grams of cabbage daily.

These references point to the good feeding qualities of oats and bran. Possibly, according to more modern standards, they may not be suitable for testing the optimum ability of animals to grow, but it would certainly be expected that guinea pigs should do very well indeed on a mixed diet containing the two, if supplemented with butter fat salts, and vitamin C.

Preliminary Preparation of Animals

Owing to the difficulty of obtaining commercially large numbers of experimental animals, which were known to have had substantially the same treatment, and which could be presumed to be standard in any degree, it was deemed advisable to subject them to a preliminary treatment. They were observed for three weeks prior to the experiment. Individual growth and food consumption records were kept. Measured amounts of strained juice of canned tomatoes and fresh spinach were fed. The tomato juice doses were graduated upward from the second day and stopped three days before the animal was used in a scurvy experi-

ment. Twenty-five cc. of tomato juice were fed the second, third, and fourth days, 50 cc. on each of the next six days, and 75 cc. on the following five to eight days. No tomato juice was fed the first day or the last three days. Fifteen gm. of fresh spinach were fed each day.

In this way, by feeding an excessive amount of vitamin-C bearing material, it was possible for the animal to have a chance to retain considerable vitamin C and at the same time to reduce any deficiency, not apparent on purchasing, which might have occurred during the first few weeks of its life. Thus, by granting the same ability or lack of ability in each animal to store vitamin C, we have at the beginning of each experiment a set of animals reasonably standard as to freedom from and resistance against scurvy.

TABLE I

EXPERIMENTAL DIETS PERCENTAGE OF INGREDIENTS

Diet	OB_1	OB_2	OB_3	YOB	1/2YOB	OBG_1	OBG_2	Kenny*
Rolled oats	60.89	52.48	40.00	24.62	42.70	56.37	42.38	39
Bran	31.39	26.73	20.00	12.31	21.23	28.88	21.68	20
Butter fat	4.95	4.95	5.00	9.85	7.40	4.92	4.92	10
NaCl	.99	.99	1.00	.99	.98	.99	.99	1.0
NaH_2PO_4, H_2O	.49	.84	1.65	—	.25	.54	1.47	—
$CaCO_3$	1.49	16.34	1.60	2.95	2.22	1.33	1.47	—
Brewer's yeast	—	—	—	49.26	24.69	—	—	—
Corn starch	—	12.38	30.75	—	—	—	21.88	—
Calf skin gelatin	—	—	—	—	—	.69	.52	—
Skim milk powder	—	—	—	—	—	—	—	30
Crude protein	14.7	12.4	9.5	32.5	22.7	20.8	14.8	20.9
Ca/P Ratio**	.14/.16	.14/.16	.14/.16	.26/.28	.20/.22	.14/.16	.14/.16	.14/.16

* This diet is described by Kenny (9).

** Ratios are given in approximate amounts (gm.) of calcium and phosphorus in 20 gm. of food, which is a maximum daily ration.

The preliminary period of three weeks served another purpose in permitting us to weed out many of those animals which had incipient or latent disease. We rejected animals which did not show normal appetites and consistent growth and subjected them to a thorough post-mortem examination. Chronic pulmonary disease or some other pathologic condition was usually found. Only experiments on male animals are reported.

THE DIETS

The composition of the diets is given in Table I. The purposes of these diets are obvious, namely, to study the effect of reduction of protein in the OB_1 diet, which contained the maximum amount of crude protein possible when using only oats and bran, as well as its possible supplementation

by other protein. In addition, the OB_1 diet listed in the first column of the table was modified and fed in a liquid or mushy state (Diet LOB) containing 20 per cent of solids and 80 per cent of tap water, or (Diet LOBTJ) containing 20 per cent of solids, 65 per cent of tap water, and 15 per cent of filtered juice of canned tomatoes. These two modifications were used to test the effect of cooking in the presence of alkali.

The LOB diet was prepared by mixing the oats, bran, and water in a large flask. Sufficient sodium hydroxide was added as N/2 solution to form the necessary amount of sodium chloride for the diet on later neutralization with hydrochloric acid. This mixture was placed in an oven held at 95° C for 2 hours, on each of three consecutive days, without causing gelatinization of starch to such an extent as to prevent ease of shaking and mixing, the flask being stoppered with a cotton plug. Between heatings the material was kept in a refrigerator to prevent spoiling. After the third heating sufficient standard hydrochloric acid was added to neutralize the sodium hydroxide present. The calcium carbonate and monosodium phosphate were also added at this point. The mixture was then cooked at the temperature of boiling water for one hour to gelatinize the starch, the melted butter-fat was added, and the diet was thoroughly mixed. This diet was fed in a negative control experiment, supplemented by brewer's yeast, and in a positive control experiment supplemented by yeast and 15 cc. of tomato juice daily. The yeast was fed by pipette as a suspension in water, 400 mg. being given daily for the first 17 days of the negative control experiment. The tomato juice was the filtered juice of canned tomatoes and was fed by pipette throughout the positive control experiment.

The LOBTJ diet was prepared exactly as the LOB diet except that 15 cc. of filtered tomato juice were used to replace a like amount of water per 100 gm. of final diet prepared. The tomato juice was added to the first mixture before any heating was done. This diet was used in a negative control experiment supplemented by yeast, as mentioned above, to ascertain whether the method of cooking caused destruction of vitamin C when the latter was known to have been present.

Results of Feeding Tests

Two sets of data were obtained, namely, those dealing with the sufficiency of these diets when fed with 15 cc. of filtered juice of canned tomatoes daily (positive controls) and when no tomato juice was fed (negative controls).

TABLE II

POSITIVE CONTROLS ON VARIOUS DIETS

Animal No.	Entering weight	Final weight	Total gain	Duration	Total food eaten	Gain per gm. food	Gain per gm. protein	Gain per day
Male	gm.	gm.	gm.	days	gm.	gm.	gm.	gm.
OB_1 Diet—14.7 per cent Crude Protein*								
620	275	496	221	85	1709	.129	.880	2.6
768	235	357	122	50	740	.165	1.12	2.4
770	225	405	180	60	1118	.161	1.09	3.0
771	300	424	124	51	921	.135	.934	2.4
779	211	332	121	60	815	.148	1.00	2.0
Average	249	403	154	61.4	1061	.148	1.01	2.5
OB_2 Diet—12.4 per cent Crude Protein*								
763	236	363	127	48	910	.139	1.12	2.6
767	252	468	216	60	1212	.179	1.44	3.6
769	259	376	117	60	999	.117	.94	2.0
776	249	363	114	51	861	.132	1.07	2.2
Average	249	392.5	144	55	995	.142	1.14	2.6
OB_3 Diet—9.5 per cent Crude Protein*								
803**	244	325	81	41	721	.112	1.18	2.0
YOB Diet—32.5 per cent Crude Protein*								
616†	320	603	283	90	2214	.127	.393	3.1
One-Half YOB Diet—22.7 per cent Crude Protein*								
618†	248	559	311	90	1787	.174	.767	3.5
OBG_1 Diet—20.8 per cent Crude Protein*								
765**	221	348	127	51	818	.155	.746	2.5
OBG_2 Diet—14.8 per cent Crude Protein*								
764	267	444	177	60	1128	.157	1.06	3.0
773	274	406	132	60	1036	.127	.86	2.2
775	250	410	160	50	915	.175	1.18	3.2
Average	264	420	156	56.7	1026	.152	1.03	2.7
Milk Powder—Rolled Oats—Bran Diet—20.9 per cent Crude Protein‡								
Average of 11 animals	329	626	297	102	2131	.138	.66	2.9

Notes to table on next page.

The positive control experiments with the OB_1 and OB_2 diets, containing 14.7 and 12.4 per cent of crude protein respectively, show that these diets are capable of producing good growth in the guinea pig. For comparison a summary is given of data obtained from the feeding of a diet of rolled oats, bran, and skim milk powder containing 20.9 per cent of crude protein, as well as from two experiments in which yeast furnished large amounts of protein. One experiment with 9.5 per cent of crude protein obtained from rolled oats and bran indicates the possibility of even this concentration being adequate. A higher concentration of protein than can be attained by using rolled oats and bran, may be expedient if one wishes to use as subjects in scurvy experiments animals whose growth capacity is being stimulated to a maximum or which have unquestionably had the best opportunity to grow. While it would seem possible for it to be only a matter of experimenter's choice, we propose, nevertheless, to consider the advisability of this supplementation in another paper.

The two experiments with dried brewer's yeast indicate that guinea pigs can do well on as much as 12 to 25 times the amount of yeast which would be supposed to furnish the optimum amount of vitamin B for a rat. Yeast, however, is not an advantageous source of protein for guinea pigs on account of their objection to its taste when used in the greater concentrations.

The experiments with gelatin as a supplement were inconclusive. Rather than attempt to work further on this intricate and involved point, we have decided to avoid this laborious task and to direct our attention in the future to protein-bearing material which when taken alone is known to be entirely adequate for nutrition.

Our experiments, like all experiments which are performed on guinea pigs, throw no final light on the question of vitamins other than the anti-scorbutic factor, since these principles are furnished simultaneously with vitamin C in green food, etc. We have but one course of action at present and that is to furnish all factors except vitamin C in apparent super-abundance.

The results of negative control experiments are given in Tables III and IV. One important result from these experiments is not indicated

* 15 cc. of tomato juice per day carried 0.1 gm. of crude protein (0.11% N).

** Only one experiment each on OB_3 and OBG_1 diets is reported as the other animals started had to be discontinued on account of respiratory disease. It became evident as the experiment progressed that their replacement would not make any essential addition to the theme of this paper.

† Only one experiment in each group is reported on account of inability to get animals to consume the yeast diets.

‡ Vitamin C furnished by 15 gm. of fresh spinach daily.

directly in the table; namely, that these tests are absolutely unreliable unless performed on animals in the best of health and condition. It is possible to be deceived, since weakling, under-vitaminized and diseased animals, when fed diets which according to our modern criteria contain traces of vitamin C, will die in the interval set for healthy animals fed diets believed to be free or practically free of vitamin C. Every investigator finds himself in this dilemma, especially when using commercial animals. Since this is the case, it is obvious that the negative control test

TABLE III

NEGATIVE CONTROLS ON VARIOUS DIETS

Animal No.	Preliminary period	Entering weight	Beginning Experimental weight	Maximum weight*	Final weight	Lived less than	Remarks
Male	days	gm.	gm.	gm.	gm.	days	
			OB_1 Diet—Small Animals				
615	17	264	304	342(16)	210	27	
619	17	237	278	346(15)	175	33	
621	17	249	294	368(18)	184	29.5	
622	17	236	324	354(18)	188	32.5	—Small patch of atelectasis in both apices
624	17	262	338	353(16)	175	28.5	
625	17	242	314	327(10)	205	28.5	
628	17	245	291	355(13)	175	28.5	
627	17	247	270	369(14)	192	29	—Small patch of atelectasis
629	17	230	228	298(12)	175	26	
Average		246	293	346	187	29.2 ± 2.0	—40% loss from beginning experimental weight.
			OB_1 Diet—Large Animals				
602	22	326	490	514(18)	275	34	
605	22	330	426	436(11)	218	28.5	
607	22	355	465	495(14)	274	29	
608	22	384	458	471(14)	239	31	
609	19	341	442	448(13)	212	28	
610	19	342	432	466(18)	245	30	
611	19	353	402	433(14)	240	30.5	
612	19	360	462	456(15)	244	32.5	—Small patch of atelectasis
613	19	330	462	471(15)	238	30	
614	19	342	400	415(14)	213	29	
Average		346	444	460	240	30.2 ± 1.8	—45% loss in beginning experimental weight.

* Numbers in parenthesis refer to day of obtaining maximum weight.

does not serve two purposes simultaneously, namely, that of a test of quality of animals and that of quality of feeding materials.

The proper future and practical application of this test will undoubtedly take cognizance of rigid minimum as well as maximum survival times. Finding the absolute definition of a diet free from vitamin C, it would be expedient to select those raw materials which as purchased are likely to be most free from this vitamin, or, in the conventional terms of the day, are free from detectable amounts of it.

Two sets of animals were used on the negative control experiment or

TABLE IV

NEGATIVE CONTROLS ON VARIOUS DIETS

Animal No.	Preliminary period	Entering weight	Beginning experimental weight	Maximum weight*	Final weight	Lived less than	Remarks
Male	days	gm.	gm.	gm.	gm.	days	
				LOB_1 Diet, Cooked in Alkali			
737	21	275	376	416(11)	218	31	400 mg. of yeast daily for first 17 days of the experiment by pipette.
745	21	285	371	413(6)	237	22.5	
740	21	286	378	416(14)	255	27	
743	21	283	377	430(16)	236	30	
Average		282	375	418	236	27.6	Average loss in beginning experimental weight 27 per cent.
				Full and One-Half Yeast Diets (YOB and 1/2 YOB)			
604	22	326	366	356(18)	242	29	Appetite fair to good. YOB diet.
599	22	308	327	350(21)	161	30	Refused YOB. Fed 1/2 YOB. beginning 4th day.
626	17	248	302	374(18)	180	31.5	1/2 YOB diet.
Average		294	332	360	194	30.2	Average loss in experimental weight 48 per cent.
				Gelatin (OBG_1) Diet			
643	22	278	371	383(7)	192	26	Six other rejected animals showing chronic lung disease lived on an average of 27.75 days.
669	22	285	370	406(16)	200	32	
670	22	280	334	352(14)	199	27	
Average		281	358	380	197	28.3	Average loss in experimental weight 45 per cent.

* Numbers in parenthesis refer to day of obtaining maximum weight.

the OB_1 diet. The larger animals, weighing 400–500 gm., showed a survival time of 28 to 34 days with a mean of 30.2 days and a standard deviation (10) of ± 1.8 days. The smaller animals, weighing 250–350 gm., showed a survival time of 26 to 33 days with a mean survival time of 29.2 days and a standard deviation of ± 2.0 days.

We cannot say but that the survival times might ordinarily be slightly shorter by a day or so than we have found, if cumulation is shown to be a factor. It is improbable that experimenters would obtain animals which had had an opportunity to acquire vitamin equal to that of animals herein referred to, although it is still possible that cumulation may not be a factor.

The series of experiments with the LOB_1 diet cooked in alkaline solution was not a complete success on account of the rejection of many animals having lung disease, which escaped diagnosis in our preliminary scrutinizing examination. Only four unrejectable experiments were obtained. One of these is apparently of rather short duration and points to the absolute necessity for more numerous data. If this experiment be omitted, the average duration of life of the other three is 29.3 days, almost a perfect check with the animals of the same size on the uncooked OB_1 diet. Many of the rejected experiments were of a satisfactory duration but others were not, so we have followed the procedure of reporting no experiments on animals with certain lung disease. The several experiments without yeast for controlling the destruction of the antineuritic vitamin, those for controlling the effect of alkali on the protein, as well as those designed to show that the method was susceptible of destroying added vitamin C, are not reported. The results were satisfactory enough, on the whole, to indicate that the test was fair and that there was at least no appreciable vitamin C in our rolled oats and bran. A similar series of tests will be run again later on the rolled oats-bran diet, supplemented with other protein.

The three negative control experiments with yeast are in agreement with the supposition of Cohen and Mendel (11) as well as with Hess (7) that our sample of yeast contained no detectable amounts of vitamin C. Yeast has also been used in scurvy diets by Parsons and Reynolds (12) and by Bertha Clow* with apparent success. We fed 12 to 25 times the amount that ordinarily might be used for furnishing vitamin B to a rat, and we believe that a few experiments of this type are much more conclusive for showing up traces of vitamin C than more numerous ones in which only the amount of yeast to be used regularly is administered.

Gelatin, which from its method of manufacture by the lime water pro-

* Personal communication to the authors.

cess, would not be expected to contain vitamin C, was found not to contain detectable traces of this factor.

Summary

A diet containing 14.7 per cent of crude protein, obtained from rolled oats and bran in the ratio of 2:1, butter fat and salts, has been described. This is about the maximum amount of dietary protein possible to obtain by using these raw materials. It would appear that this diet is perfectly satisfactory for the nutrition of guinea pigs, and that it contains considerably more than a minimum of the known essential amino acids. The survival time on this diet alone of guinea pigs standardized by previous preliminary feeding of large amounts of vitamin C, has been found to be 28 to 34 days (average 30.2 ± 1.8) for animals weighing 400–500 gm., and 26 to 33 days (average 29.2 ± 2.0) for animals weighing 250–350 gm.

Some data obtained with this diet, when it was cooked in alkaline solution previous to feeding, indicate that it contains no detectable amount of vitamin C. It appears not improbable that it contains absolutely none.

A few preliminary experiments with dried brewer's yeast in large concentrations indicate that it is possible to use this material as a bearer of vitamin B in scurvy diets without fear of adversely affecting the guinea pigs or without the introduction of detectable amounts of vitamin C. We would not recommend it in large concentrations as a protein supplement on account of the taste.

The diet of oats and bran would appear to be eminently suited for animals placed in acute qualitative tests of materials which contain only traces of, or no, vitamin C. It would probably serve for the longer 90 day tests except that, for the present, expediency rather than demonstrable necessity would dictate that we supplement it with both protein and vitamen B complex. In view of the difficulty and uncertainty of obtaining final convincing evidence that would entirely suffice, it will be better in the future to direct attention to such supplementation.

Attention has been called to the unreliability of the negative control test and the possible fallacies in the results therefrom. It would be well for the investigator, if he is inclined even by tacit implication to insist on a diet free of vitamin C, to avoid the dilemma of being unable to prove from his tests whether the diet or the animals he is using are satisfactory. It is possible that at an early date this test may revert to its proper status, namely, one of animal rather than dietary control. Even though it is logically to be presumed that all commercial rolled oats, bran, and dried brewer's yeast are free of disturbing amounts of vitamin C, we would nevertheless

recommend a test of such materials before using as well as the preparation of the diets from large uniform batches of raw materials.

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NERVE DEGENERATION RESULTING FROM AVITAMINOSIS A

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BERI BERI, scurvy, and rickets were each recognized as a definite disease characterized in each case by well known symptoms and pathological lesions, long before the corresponding vitamins, vitamins B, C, and D, were discovered. The discovery of these vitamins served merely to clear up the etiology of these well known diseases. Very little information concerning the symptoms and pathology of these diseases has been added since it was discovered that they resulted from the lack of these vitamins.

This is not the case, however, with vitamin A. This vitamin was discovered as a result of the study of an eye disorder which was found to develop in experimental rats on a somewhat restricted diet. Aside from this eye trouble which occurred in experimental rats, no definite disease was known for which this vitamin could be considered as the etiological factor. For this reason it has been necessary to study the tissue changes which result when vitamin A is lacking in the diet. A number of investigators have reported the results of their studies of this subject.[1] The most extensive of these studies have been reported by Mori (1922) and Wolbach and Howe (1925).

The outstanding pathological change reported by these workers seems to be a keratinization of the epithelium throughout the body. This change in the epithelium lowers the resistance to bacterial infection, which results in secondary lesions. As a general rule in the last stages of avitaminosis A these secondary lesions, resulting from infection, overshadow the primary changes brought about as a direct result of the lack of the vitamin. In the rat the eye and surrounding tissues are almost always involved.

In no case, so far as we are aware, has anyone reported the finding of pathological changes in the nervous system accompanying avitaminosis A. Wolbach and Howe (1925) have the following to say concerning the influence of the lack of this vitamin on the nervous system in the rat.

"The Nervous System—As the rats exhibited no symptoms pointing to nervous system lesions, the peripheral nerves were not studied. No les-

* Contribution No. 144.

[1] A good review of this work can be found in "The Newer Knowledge of Nutrition" (third edition) by McCollum and Simmonds.

ions were found in the brain, cerebellum and sympathetic ganglia. The ganglion cells of the myenteric plexus were normal whenever found."

While it is true that rats show no striking nervous symptoms accompanying avitaminosis A, it is not uncommon for those in the advanced stage of the disorder to show a distinct incoordination which suggests an involvement of the nervous system. This incoordination is very slight, however, in the case of rats. With swine, we have found the incoordination to be the outstanding symptom of avitaminosis A, while the eye lesions, which are so prominent in the rat, are of little importance.

In our studies (1928) of the importance of vitamin A for swine, six lots, including twenty-seven individuals, have been fed a ration which is deficient in vitamin A. This ration consisted of white corn, tankage, and bone ash. Complications from rickets were avoided by allowing the pigs an outside yard where they received an abundance of direct sunshine. The pigs were placed on this vitamin A-deficient diet at weaning time and were continued on the diet until they died, or until they reached such an advanced stage of the disorder that they would have died in a few days, when they were killed for post-mortem examination. As it was thought that the pigs would develop eye lesions similar to those which characterize avitaminosis A in rats and chickens, careful watch was kept of their eyes. While there was more or less watering of the eyes, there were no cases of severe conjunctivitis, and in no case did there seem to be an involvement of the tissue around the eye. In one case a small corneal ulcer developed. Without exception these pigs developed a marked nervous disorder, in from six to ten months, characterized by blindness, incoordination and spasms (Figure 1).

Hart, Miller, and McCollum (1916) observed similar symptoms and nerve degeneration in pigs, in their studies on the nutritive deficiencies of wheat and grain mixtures. Since their work was done before there was a clear understanding of the cause of deficiency diseases, they attributed the pathological condition to some toxic principle in the wheat rather than to a deficiency in the diet. The beneficial action of alfalfa in preventing these symptoms was ascribed to the amount of fat-soluble A and minerals which it contained. These substances in some way offset the toxic action of the wheat.

That this nervous condition in our experiment resulted from a lack of vitamin A was shown by the fact that it could be prevented or relieved by incorporating in the feed an adequate supply of substances known to be good sources of this vitamin, such as cod liver oil, butterfat, yellow corn, and alfalfa leaf meal.

As these symptoms indicated an involvement of the nervous system, histological examinations were made of the tissue. The tissue, after being fixed by Mueller's fluid, was stained with osmic acid, employing the Marchi method. Definite degeneration of the nerve bundles was found in the optic thalamus, optic, femoral, and sciatic nerves, and certain parts of the spinal cord. Nerves from normal hogs were examined by the same method using the same solution and in no case did they show suspicious staining characteristics. The accompanying micro-photographs illustrate the type of

Figure 1.—Pigs in an advanced state of avitaminosis A, a condition characterized by extreme incoordination and spasms.

degeneration found in the nervous tissue (Figure 2). These histological findings, together with the clinical symptoms, show quite clearly that the lack of vitamin A results in the degeneration of the nervous system in the case of swine.

Other animals show similar marked nervous symptoms in the advanced stages of avitaminosis A. In studying the importance of vitamins in the ration of dairy cattle, we have fed three mature cows on a feed deficient in vitamin A[2]. While these cows did not develop spasms like the pigs on a similar feed, they did show marked stiffness and incoordination.

Young chicks resemble pigs in the severity of the nerve disorder accompanying avitaminosis A. In one group of five hundred day-old chicks placed on a vitamin A-free feed, more than ninety per cent developed symptoms showing severe nerve degeneration[3]. One not familiar with the fact that

[2] Unpublished data.
[3] Unpublished data.

lack of vitamin A causes nervous symptoms in chicks, might mistake the condition for polyneuritis resulting from the lack of vitamin B (Cruckshank, Hart, and Halpin, 1928). Histological examination is now being made on the nervous system of these chickens to see whether or not they show the same type of nerve degeneration found in pigs.

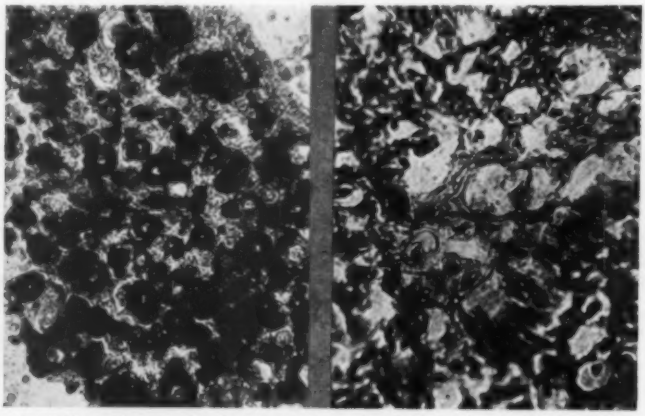

Figure 2.—Micro-photograph of cross section of spinal cord. Right hand figure, from normal cord. Left hand from cord of pigs in advanced stages of avitaminosis A. Blackened areas indicate degeneration.

Conclusions

The lack of vitamin A in the diet of pigs, chickens, and cows, results in marked nervous symptoms characterized by impaired vision, incoordination and spasms.

Histological examination of the nerves of pigs in the advanced stage of avitaminosis A, shows degeneration of some of the nerve bundles. This degeneration was observed in portions of the spinal cord, optic, sciatic, and femoral nerves.

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INFLUENCE OF FIBER ON NITROGEN BALANCE AND ON FAT IN THE FECES OF HUMAN SUBJECTS

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DOES the presence of fiber in varying amounts in the ordinary diet affect the utilization of the nutrients? This question presented itself to the authors of this article when comparison was made of the results of two balance experiments with human subjects (1, 2) done in the Nutrition Laboratory of the University of Chicago. The two junior authors (A. W. and J. W.) each served as a subject in her own experiment, and one of them (J. W.) was a subject also in the other's study. The experimental diets, planned to meet present day criteria of adequacy, contained liberal, though not unusually large, amounts of fiber. The coefficient of digestibility of nitrogen in these two studies was found to be distinctly and consistently lower than the accepted average figure for mixed diets, (85 to 87 in one case and 88 to 89 in the other *vs.* the usual 92), while the coefficient of digestibility for fat, as determined in one of the Chicago experiments, even exceeded the accepted average figure (97.4 to 98.2 *vs.* the accepted 95). Also, of the two Chicago diets the one with the higher fiber gave the lower figure for the nitrogen.

The influence of fiber on the utilization of nutrients may have significance in problems of animal feeding as well as in human nutrition.

Previous Work

The extensive literature, which has accumulated on the subject of the digestibility of foods, contains comparatively little comment upon the influence of fiber on the utilization of the nutrients. But fiber has usually been included in experimental diets whether they were very simple, as in earlier days, or more complex, as in recent times.

In the numerous and valuable studies of the digestibility of particular foods made in the U. S. Department of Agriculture, the diets were deliberately kept very simple in order that the effect of the specific food might be apparent. For example, one may mention the studies of Woods and Merrill

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** Now on the staff of the Department of Household Science, University of Toronto.

(3) with bread in 1900; Doane (4) with cheese in 1911; Langworthy and Holmes (5, 6, 7, 8) with certain animal fats in 1915, with grain sorghums and with very young veal in 1916, with certain vegetable fats in 1917; Holmes (9, 10) with some nut oils in 1918, and with wheat bran 1919; Langworthy and Deuel (11) with raw corn, potato, and wheat starches in 1920. The simplicity of food combinations used in the government studies served as a pattern for other investigators.

More recently the tendency in digestibility and balance experiments has been to employ a greater assortment of food materials. Typically varied experimental diets are those used by the authors of this report, by Rose and McLeod (12, 13) in their study of the digestibility of raw egg white, and by McLaughlin (14), in her comparison of spinach with milk as a source of calcium.

University of Chicago Studies—Experimental Diets

The make-up of the diets in the University of Chicago studies, in the matter of the distribution of nitrogen and calories, and with respect to details of similarity and difference between the two diets, is shown in Table I.

The whole milk powder in J. W.'s diet was diluted so as to be the equivalent of cow's whole milk. In period 1 for A. W.'s diet, the milk was fresh pasteurized whole milk; in period 2, evaporated milk diluted to be the equivalent of cow's whole milk.

Calories provided by J. W.'s diet were approximately 2300 per day and 1945 in A. W.'s meals. Fuel values in J. W.'s were calculated from the percentage compositions found by analyses, and the calorific factors, protein 4.35, fat 9.45, carbohydrate 4.1, were not reduced for loss in digestion, since coefficients of digestibility were determined in this study. Fuel value for A. W.'s diet was calculated from figures in Sherman's tables (15).

The daily intake of the two subjects on A. W.'s diet varied only in that J. W. ate 100 grams of lettuce and 300 grams of peaches, while A. W. had but 30 grams of lettuce and 240 grams of peaches.

Experimental Procedure

Each experimental period covered six days. Except for water and salt, all food portions eaten during each period were carefully weighed on a trip scales, and the food was consumed as nearly quantitatively as possible.

Samples of the food eaten on the last three days, also urine and feces for the same period, were analyzed.

For determination of nitrogen in urine, the micro-Kjedahl method of

TABLE I

DISTRIBUTION OF NITROGEN AND CALORIES IN THE DAILY DIET

Foods	A.W.'s Diet					J.W.'s Diet						
		Period 1		Period 2			Period 1		Period 2		Period 3	
	Intake Gm.	N. gm.	Total Energy Calories	N. gm.	Total Energy Calories	Intake gm.	N. gm.	Total Energy Calories	N. gm.	Total Energy Calories	N. gm.	Total Energy Calories
Beef	100	3.14	155	3.17	155	135	4.73	161	4.47	202	4.47	159
Milk	133	0.74	91	0.78	91							
Dry Milk†						81	2.94	441	3.40	444	3.40	444
Bread	185	2.32	485	2.36	485	135	1.88	364	1.74	366	1.78	373
Potato	360	1.11	300	1.28	300	400	1.06	424	1.39	424	1.39	320
Butterfat	40	—	360	—	360	63	—	595	—	595	—	595
Sugar	8	—	32	—	32	40	—	164	—	164	—	164
Lettuce	100	0.12	19	0.13	19							
Celery						170	0.33	39	0.20	27	0.26	29
Apple, (pared)						300§	0.11	195	0.10	162	0.11	180
Peaches (canned)	300	0.24	138	0.23	138							
Orange juice	100	0.13	45	0.13	45							
Cereal (wheat endosperm)	28	0.50	100	0.50	100							
Bacon	35	*0.59	220	*0.59	220							
Totals		8.89	1945	9.17	1945		11.05	2383	11.30	2384	11.41	2264

† Spray process.

§ 100 grams eaten raw; 200 grams cooked.

* Calculated from Sherman.

Koch and McMeekin (16) was used, and a modification of it (17) for nitrogen in food and feces. In J. W.'s experiment ether-soluble substances in meat, potatoes, apples, celery, and feces were determined by the Soxhlet extraction method; in bread by the tentative A.O.A.C. method (18); and in the powdered whole milk by Jephcott's modification (19) of the Werner-Schmidt method. Coefficients of digestibility were calculated by the common equation: $\frac{\text{grams ingested} - \text{grams in feces}}{\text{grams ingested}} \times 100 = \text{percentage absorbed.}$

Results and Discussion

The course of nitrogen and fat metabolism is shown in Table II. In each period both subjects were in practical nitrogen equilibrium. Approximately one gram of protein per kilogram of body weight per day had been eaten in each case.

The figures for coefficient of digestibility of nitrogen are striking in being almost identical for the two subjects when on A. W.'s diet, thus illustrating similarity of digestion and the assimilation process in different individuals. The same thing was seen with three of the five adult subjects in J. W.'s experiment (1), the coefficients for the two persons assimilating the same percentage of nitrogen as did J. W., being 87.7 and 86.1 respectively. The remaining two subjects had somewhat lower nitrogen coefficients, but agreed with each other, 82.5 and 83.1.

Although the fiber content of neither diet was determined by analysis or calculation (figures for the fiber content of cooked apple, and canned peaches have not been found in the literature), it is thought that J. W.'s diet contained a greater amount of indigestible fiber than did A. W.'s. According to Bulletin No. 28 and Circular No. 50, U. S. Department of Agriculture, celery contains 0.98 per cent fiber and lettuce but 0.7 per cent. The figures for raw apples are 1.0 per cent fiber, and for raw peaches 0.6 per cent. It is true that the difference in the amount of celery eaten by J. W. on her own diet (170 grams) as compared with lettuce in A. W.'s diet (100 grams) is the same as the difference in consumption of lettuce by J. W. and A. W. on the latter's diet (100 grams *vs.* 30 grams); but the actual amount of cellulose would be proportionately higher in the celery and the coarser vascular bundles of celery suggest a greater resistance to digestion than do the more uniformly tender leaves of lettuce. One third (100 grams) of the pared apples in J. W.'s diet was eaten raw, the remaining 200 grams baked; the entire 300 grams of peaches in A. W.'s diet was the canned fruit.

TABLE II

UTILIZATION OF NITROGEN AND FAT

Subject	Diet	Period	Nitrogen							Fat				
			Intake per day	Outgo—Av. Daily			Balance	Absorbed	Coefficient	Intake per day	Outgo in feces		Balance	Coefficient
				In Urine	In Feces	Total					3-days	Av. daily		
			Gms.	Gms.	Gms.	Gms.	Gms.	Gms.		Gms.	Gms.	Gms.	Gms.	
.W.	J.W.'s	1	11.05	9.77	1.65	11.42	−0.37	9.40	85.1	93.6	4.90	1.63	92.0	98.2
		2	11.30	9.26	1.45	10.71	+0.59	9.85	87.2	99.0	7.63	2.54	96.5	97.4
		3	11.41	9.85	1.50	11.35	+0.06	9.91	86.9	95.5	7.49	2.50	93.0	97.4
	A.W.'s	1	8.88	7.89	0.99	8.88	0.00	7.90	88.8					Not determined
		2	9.17	8.00	0.99	8.99	+0.18	8.18	89.0					
A.W.	A.W.'s	1	8.53	7.88	0.96	8.84	−0.31	7.58	88.8					Not determined
		2	9.00	7.53	1.08	8.61	+0.39	7.92	89.0					

With respect to the influence of cooking upon cellulose, Woodruff (20) has recently reported evidence in experiments with rats "that cooking of potato cellulose diminished the amount of it which could be recovered in the feces, no matter whether the cellulose was fed as potato itself or as fiber added to the basal ration. When a raw basal ration with raw potato fiber was fed, 58 per cent of the cellulose fed was recovered in the feces. Cooked potato fiber with the basal ration allowed for only a 31 per cent recovery. The raw potato diet showed a recovery of 83 per cent of the fiber, whereas cooked potato showed only 60 per cent." On this evidence, then, the greater amount of raw fiber in J. W.'s diet might reasonably be expected to interfere more with digestion than did the cooked fiber of A. W.'s diet.

While both diets in the Chicago studies were entirely satisfactory to maintain the habitual rhythm of bowel evacuation in the subjects, the effect of the greater bulk in J. W.'s diet seems to be shown in the slightly but consistently lower coefficient of digestibility for nitrogen in J. W. when on her own diet, than when on A. W.'s diet (85.0, 87.0, 86.9 *vs.* 88.8 and 89.0), and more clearly in the 50 per cent higher fecal nitrogen of J. W., 1.65, 1.45, 1.50 gms. on her own diet, as contrasted with 0.99 and 0.99 on A. W.'s diet.

An interesting contrast to the effect of a liberal amount of fiber upon the utilization of protein, is shown by the fact that the coefficients of digestibility of fat for J. W. upon her own diet, 97.4, 98.2, 97.4, are slightly higher than the usually accepted figure, 95.

With these observations, McLaughlin's (14) findings are in accord, for when relatively large amounts of spinach, 250 to 325 grams per day,* replaced milk as a source of calcium in her experimental diet, the average coefficient for protein for seven subjects fell from 91.9 on the milk-containing diet to 88.6 on the spinach-containing diet. One subject in her experiments had the same nitrogen coefficient on the spinach-containing diet as on the milk-containing diet. When the figures for the remaining six subjects are averaged, the nitrogen coefficient on the spinach-containing diet becomes 87.4, and the milk-containing diet 92.8. The average coefficients for fat in McLaughlin's experiment are the same on the two diets, 97.2 and 97.7, and are slightly higher than the accepted average coefficient for fat.

Another interesting similarity between McLaughlin's study and these done in the University of Chicago is seen in the water content of the feces. The following percentages of water are reported: for McLaughlin's

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* Thanks are here expressed to Dr. McLaughlin for use of data from her private files.

two periods 80 and 82 respectively; for J. W. on her own diet 80; for J. W. on A. W.'s diet 80.5; and for A. W. on her own diet 80.6.

Woods and Merrill (3) in their tests of digestibility of white, entire wheat, and Graham bread when each was eaten with milk, found these coefficients:

	Protein	*Fat*
Milk and white bread	93.6	95.5
Milk and entire wheat bread	91.7	96.5
Milk and Graham bread	88.6	95.5

While the percentage of available nitrogen decreases with the increase of fiber in the diet, the availability of the fat remains practically the same. Blunt and Mallon (21) in their study of the digestibility of bacon used an average amount of 119 grams of much-cooked or 158 grams of slightly-cooked bacon, together with large amounts of shredded wheat (390 to 490 grams) and 1080 cc. to 1450 cc. of orange juice and a little sugar as a daily diet. They found the average coefficient for fat to be 92.8 when the bacon was slightly cooked, and 95 when much cooked, thus agreeing with the usually reported figures. The corresponding average nitrogen coefficients were 79.8 and 80.0. These authors comment: "The nitrogen of the diet was not so well utilized. These low figures, however, are undoubtedly due not to the bacon nitrogen but to the shredded wheat the coefficient of digestibility of which has been reported to be 57.7." Similarly, Holmes' (10) study of the digestibility of wheat bran, when eaten along with potato, apple, orange, butter and sugar, showed an average coefficient of 93.1 for the fat of the entire diet, whereas the coefficient for the protein was very low, being but 45.3, when the cases of negative nitrogen balance are excluded in calculating the averages. The uniformly high coefficients found for both vegetable and animal fats (with two exceptions 95 or above), in the extensive series of tests made in the Department of Agriculture, also testify to the non-interference of the fiber (from wheat biscuit, apple and orange) with the utilization of fats in a mixed diet.

These results in human studies corroborate Mitchell's (22) findings upon the effect of roughage on the fecal nitrogen of rats. He fed four litter mate rats a nitrogen-free diet. In one test the rats were allowed to eat filter paper in addition to the diet. With paper in the diet the fecal nitrogen was 180, 164, 159, 191 mg.; without paper 124, 111, 130, 123 mg. respectively for the four rats. The average of 173 mg. fecal nitrogen excreted when filter paper was consumed, is an increase of 42 per cent over the average of 122 mg. fecal nitrogen on a nitrogen-and-fiber-free diet.

The lower-than-average coefficient for nitrogen in diets which are high

in fiber may have two possible explanations, singly or in union. The large amount of fiber may interfere with the action of proteolytic enzymes; the diet, as a whole, may occasion an unusually large outgo of metabolic nitrogen.

Summary

It may be concluded on the basis of the data from the experiments which are drawn upon for this report, that a high proportion of bulk in the diet tends to reduce the utilization of nitrogen to a lower plane, but that it does not have the same effect upon the utilization of fat in the diet.

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THE NUTRITIVE VALUE OF NEW ZEALAND SPINACH

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NEW ZEALAND spinach (*Tetragonia expansa*) is a comparatively new kind of market greens. Its particular advantage is in its being heat tolerant. In the hot summer months when spinach and some other green vegetables cannot be grown, New Zealand spinach, which is not a variety of spinach (*Spinacia oleracea*), is easily grown as a truck crop and furnishes succulent greens for months. Furthermore it is being grown as a winter crop in some eastern greenhouses. The tender, rather fleshy leaves have a distinct flavor more pleasing to some than that of common, mild spinach.

The nutritive value of any vegetable used as a salad or for greens is dependent largely on its mineral and its vitamin contents. The few references to New Zealand spinach in the literature (3, 7) show it to be high in minerals, especially iron. No references were found to its vitamin content. Its appearance, flavor and composition, as found in our experimental work, are recommendation enough. It is compared with spinach only because this has previously been shown to be exceptionally rich in minerals and potent in vitamins. The use of spinach has been advocated to such an extent that recognition of other vegetables with different flavors is needed before unfavorable reaction towards eating spinach becomes too great.

Mineral Content

Raw and cooked samples were analyzed for moisture, total ash, calcium, phosphorus and iron. Leaves alone were used for analysis because much of the stem, particularly in older plants, is tough and fibrous. Samples of New Zealand spinach, as gathered in the college greenhouse or garden, showed no dirt visible to the eye and washing yielded a negligible amount of foreign matter. Therefore, the raw vegetable was analyzed without washing. It was weighed as soon as possible after picking and put into a vacuum oven,

* Spinach and New Zealand spinach were grown and furnished through the courtesy of Dr. E. S. Haber. Most of the total ash, calcium and phosphorus determinations were made by Carrie Hodges, some of the vitamin A determinations by Lena Gilbert and some of the vitamin B determinations by Iva Mullen, in partial fulfillment of the requirements of each student for the degree of Master of Science.

set at 70° C, to get the moisture content. The dried material was ashed in an electric muffle furnace at dull red heat. The ash for calcium and phosphorus determinations was dissolved in hydrochloric acid as described by Clark (2). Calcium was precipitated as the oxalate in the presence of acid (pH 4.8) and determined by titration against permanganate (9). Phosphorus was determined by a modification of the Pemberton-Kilgore method (6), precipitating the phosphomolybdate, dissolving this in standard sodium hydroxide, and back titrating. Iron was determined colorimetrically by the Walker method (4).

As shown in Table I, raw New Zealand spinach is exceptionally high in mineral salts, is a very good source of iron and of calcium but not of phos-

TABLE I

PERCENTAGE COMPOSITION OF NEW ZEALAND SPINACH LEAVES[4]

	Water	Water	Ash	Ash	Ca	P	Fe	Protein (Nx6.25)	Oxalic acid, anhydrous
Av.	94.0[1]	88.4[2]	1.92[1]	2.43[2]	.045	.027	.0023	1.4	.49
P.E.[3]	0.3	1.1	0.01	0.05	.002	.002	—	0.2	.03
Max.	95.0	89.8	1.94	2.58	.050	.030	.0024	1.7	.53
Min.	93.3	85.6	1.90	2.38	.038	.022	.0022	1.1	.40
No. anal.	18	11	5	6	10	6	5	8	8

[1] Samples grown in greenhouse.

[2] Samples grown in garden.

[3] Probable error of the mean.

[4] Lichtin (7) gives the following percentages: 1.86 ash, and 0.00456 iron, in New Zealand spinach grown at Cornell; 2.59 ash, and 0.00328 iron, in samples from the open market.

phorus. These facts are brought out by comparison with other common leafy vegetables shown in Table II.

Changes in mineral content caused by cooking are brought out in Table III. The greens were prepared for cooking in the usual way and two methods of cooking were employed. First, the weighed leaves were washed in tap water and cooked in the clinging wash water only in the top of an enamel double boiler; second, the weighed sample, after washing, was put into a measured volume of boiling tap water and cooked for a definite period. In each case the cooked product was drained and both the spinach and the cooking liquor dried and analyzed. Mineral contents were calculated on the basis of the fresh raw weight.

New Zealand spinach cooked without added water retains its distinctive

flavor with a somewhat astringent after taste. Fifty minutes in a double boiler gave a mushy product, thirty-five minutes a tender one though the brilliancy of the green color was lost. One-sixth of the mineral salts, including one-eighth to more than one-fourth of the iron, was drained off

TABLE II

PERCENTAGE COMPOSITION OF SOME LEAFY VEGETABLES

	Water[2]	Ash	Ca[3]	P[3]	Fe	Oxalic acid, anhydrous
Cabbage	91.5	1.0[1]	.045	.029	.0011[3]	.0056[5]
Lettuce	94.7	0.9[1]	.043	.042	.0007[3]	trace[4]
Spinach	92.3	2.1[1]	.067	.068	.0036[3]	.299[6]
		1.41[2]			.0061[3]	
					.0031[4]	

[1] Atwater and Bryant (1).
[2] Lichtin (7).
[3] Sherman (11, appendix B).
[4] Spinach grown under same conditions as New Zealand spinach of our analyses.
[5] Floyd, L. P., Unpublished Master's Thesis, Univ. of Chicago, 1923.
[6] Arbenz, E., *Mitt. Lebensm. Hyg.* 1917, VIII, 98.

TABLE III

COOKED NEW ZEALAND SPINACH LEAVES

	Wt. of raw sample	Vol. of water	Time cooked	Green color	Flavor	Percent Mineral Losses calculated on basis of 100 g. raw			
						Ash	Ca	P	Fe[2]
	gr.	cc.	min.						
A[1]	150	*	50	olive	very strong	28.4	5.1	—	
B	150	*	35	"	Strong	15.6	4.5	—	
C	420	*	35	"	"	14.8	4.7	5.8	13
D	420	*	35	"	"	15.6	4.5	5.5	28
E	420	*	35	"	"	15.7	5.1	5.8	28
A	150	500	20	bright	Mild	44.8	6.1	—	
B	150	150	14	"	"	41.9	6.1	—	
C	140	750	10	"	"	39.8	6.1	63.4	37
D	420	750	10	"	"	40.6	5.9	62.9	40
E	420	750	10	"	"	40.5	6.8	62.4	44
F	420	750	10	"	"				47

* Water clinging to leaves from washing.
[1] Samples A, B and D were drained 10 min., C, 60 min.; and E, 5 min.
[2] Iron was not determined in the same samples as those used for analyses of other minerals. All samples for iron determinations were drained for 10 min.

in the strong cooking liquor but little of the calcium or phosphorus was lost.

Ten minutes cooking in excess boiling tap water gave an attractive tender product with a very mild flavor. Draining away the liquid took with it little calcium but about 40 per cent of the total mineral salts and of the iron, and more than 60 per cent of what phosphorus the raw vegetable contained.

Using our figures for composition of New Zealand spinach, and average composition figures of Atwater and Bryant (1) and of Sherman (11) for the other common leafy vegetables, New Zealand spinach, even when cooked in a large volume of water and drained longer than is customary before serving, is found to be as rich in mineral salts as raw spinach and to be richer in iron and as high in calcium as lettuce or cabbage served raw.

Vitamin Potency

Only raw leaves were tested for vitamin content. Albino rats from our stock colony were used for most determinations. The diet of the mothers of other rats used had been similar, that is, largely grain with milk added. Our stock diet is Steenbock's (14) modified to contain wheat germ and yeast, and supplemented at intervals by lettuce. The laboratory technic was that of Ferry (5). Rats were weaned at 28 or 29 days and placed directly on the experimental diet in individual cages. The basal diet consisted of purified casein 18 per cent, dextrinized cornstarch (8) 76 per cent, salt mixture (10) 4 per cent, and agar 2 per cent.

Vitamin A. For vitamin A tests the casein was made A-free (12), the diet irradiated and a salt spoonful of yeast, weighing approximately 0.4 gram, was fed separately six times a week. The method used was essentially that of Sherman and Munsell (12). Cessation of growth was the chief criterion for deciding when the store of vitamin was depleted. The basal diet was then supplemented during the 8-week period with carefully weighed quantities of New Zealand spinach or spinach leaves (Bloomsdale) having, so far as possible, an upper surface area of 4 to 5 square inches. To minimize errors in weighing, the very small quantities of leafy tissue were fed three times a week. Twenty-six negative controls, with an average weight of 50 grams at 28 days, survived 53.5 days on the average or 21 to 22 days after the average depletion period.

Close to 90 mg. of New Zealand spinach per week were required to allow a gain in weight of approximately 3 grams a week for 8 weeks, after depleting our rats which at 28 days weighed on the average about 50 grams. A slightly smaller quantity of ordinary spinach grown under the same

conditions induced a somewhat larger gain with a correspondingly greater consumption of the basal diet. However, it is clearly shown that New Zealand spinach, like spinach, is several times as rich in vitamin A as are most green foods that have been tested.

Vitamin B. The method used was essentially that of Sherman and Spohn (13). Vitamins B and G were considered as an entity. The same

TABLE IV
VITAMIN A TESTS

Amt. per wk.	No. of rats	Wt. at 28 days.	Pre-period	Initial wt.	Gain in period	Food eaten weekly	Rats surviving period
New Zealand Spinach							
gr.		gr.	days	gr.	gr.	gr.	no.
0.09	16	52	33	127	27.4	66	11
0.08	15	50	30	109	16.8	52	12
0.07	15	49	35	119	−11.6	54	8
Spinach							
0.07	7	46	30	125	32.5	69	7

TABLE V
VITAMIN B CONTENT OF LEAVES

Amt. per day	No. of rats	Initial wt.	Weekly change in wt.	Food eaten weekly	Time of survival	Rats surviving period	Gain in wt. of survivors
New Zealand Spinach							
gr.		gr.	gr.	gr.	days	no.	gr.
1.0	7	50	−1.5	24.6	41	1	8
1.2	7	47	−0.4	25.6	49	4	2
1.4	8	50	−0.3	27.3	49	4	10
1.6	8	37	+3.3	21.4	56 (K)	8	26
Spinach							
1.0	8	49	+2.5	32.6	50	6	29
1.2	10	49	+3.4	34.9	56 (K)	10	27
1.4	7	37	+6.5	35.8	56 (K)	7	52

K denotes killed.

basal diet was used as for vitamin A determinations except that it was not irradiated and the casein was made B-free (13). Cod-liver oil, five drops per day, was fed separately. The New Zealand spinach and the spinach was the same as for vitamin A tests but was fed six times per week with a double portion on Saturdays. Fifteen rats, with an average weight of 46 grams at 28 days, used as negative controls, survived for 40 days on the average, with an average loss of 2.6 grams in weight per week.

It requires a little more than 1.4 grams of New Zealand spinach daily to maintain a 50 gram rat at constant weight for 8 weeks On 1.6 grams daily, 37 gram rats make a distinct gain, but just one-half as great as that made by rats of the same weight getting 1.4 grams of spinach (*Spinacia oleracea*). The latter rats showed an appetite for the basal diet one-half again as great. New Zealand spinach, though it contains somewhat less vitamin B complex than does spinach, belongs in the same class with it.

Summary

New Zealand spinach is especially valuable in the diet because of its high salt content. It compares favorably with most green vegetables in iron and calcium.

Cooked without addition of water, it retains most of its minerals.

If greens of milder flavor and brighter color are preferred, it may be boiled in water with little loss of calcium but with much loss of other minerals. However, it still retains enough to compare favorably with raw cabbage or lettuce.

New Zealand spinach is exceedingly rich in vitamin A and is a good source of vitamin B complex.

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CYCLIC VARIATIONS IN THE BASAL METABOLIC RATE OF WOMEN

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THE various workers who have from time to time reported experiments dealing directly or indirectly with the effect of the menstrual cycle on the metabolic rate of women have often differed profoundly in their conclusions. Among the chief investigators who have concluded that there was no demonstrable effect may be listed Zuntz (1906), Gephart and DuBois (1916), Blunt and Dye (1921), Wiltshire (1921), and Lanz (1924). Gephart and DuBois deal with the subject only indirectly. They report three tests on a single female subject so that it is not at all surprising that they failed to show an effect. Blunt and Dye's fourteen subjects actually averaged 1.6 per cent lower in their metabolic rate during menstruation than at other times but these investigators point out that variations of this magnitude may occur at any time without apparent cause.

The one point upon which there seems to be the most general agreement is the existence of the so-called premenstrual rise. An increased metabolic rate which occurred sometime during the week preceding the onset of menstruation has been emphasized by Snell, Ford and Rowntree (1920). These investigators found that the rise in the metabolic rate often occurred during the catamenia itself. Other investigators who have noted a similar rise in the metabolic rate of their female subjects are Rowe and Eakin (1921) and Wakeham (1923).

A decrease in the metabolic rate occurring on the first or second day of menstruation has been noted by Kunde (1923), Hafkesbring and Collett (1924), and Collett and Liljestrand (1924). Collett and Liljestrand found that the metabolic rate reached a minimum on the first or second day of menstruation and that there was a second low point ten to eighteen days later.

Quite recently Rogers and Flemming (1928) have reported experiments on seven normal women from which they conclude that there is "a relation between the metabolic rate and the menstrual cycle with the lowest point in the metabolic cycle coming during or immediately following the menstrual period." Another recent paper is that of Benedict and Finn

(1928). They have published valuable and interesting data on the gaseous metabolism of one female subject over a period of years. With this subject the average oxygen consumption during menstruation was 3 per cent lower than for the inter-menstrual periods. In evaluating their data the authors emphasize the unusually good physical condition of this subject. Still more recently Griffith (1929) and his coworkers have published data which include observations on the basal metabolism of three women over a period of one year. All three of these women showed a cyclic variation in the metabolic rate which was synchronous with the menstrual cycle. However, the individual cycles were not identical. One subject showed the lowest rate during menstruation with a progressive rise during the intermenstrual period. The remaining two subjects showed the lowest rate in the second or third week of the menstrual cycle. All three, however, showed an increased metabolic rate during the third or fourth week of the cycle.

When one considers this mass of conflicting evidence it appears that the only hope of reaching satisfactory conclusions lies in the accumulation of sufficient data to justify statistical treatment. With this idea in mind we began, some three years ago, an investigation of the day-by-day variations in the basal metabolism of women. The apparatus used was the Benedict-Roth-Collins apparatus with kymograph attached. All calculations were made from the graphic record of the respirations of the subject. In general the technic was that described and recommended by Roth (1923). During the course of the investigation more than eight hundred basal metabolism determinations were made on twenty-seven women. In the final analysis of data only those subjects who were under observation for at least two complete menstrual cycles were included. This rule resulted in the elimination of six subjects. One additional subject was ruled out on account of her rather erratic conduct. It was felt that she was not cooperating as fully as could be desired and that the tests made on her were, therefore, not altogether reliable. This left twenty subjects in the final compilation of data, upon whom a total of 625 basal metabolism determinations was made. Of these, 155 tests were made during menstrual periods; the remaining 470 between menstrual periods. All tests were run in duplicate and the lower one used. This was done on the supposition that many things might happen to a subject that would increase her metabolic rate but that aside from sleep nothing could happen that would decrease it. In no case did any of the subjects fall asleep during a test. The average difference between the duplicate tests was less than three per cent.

It was usually found that the tests run on the first day any particular subject came to the laboratory gave results appreciably higher than those obtained in subsequent tests. Therefore, tests made on the first day were in every case discarded. In a few cases it was found necessary to discard the test made on the first two or three days since they were well above the metabolic rate which subsequent tests showed to be normal for the subject. This observation would indicate that it is rarely, if ever, safe to base scientific or clinical findings on a single basal metabolism test. This point has also been emphasized by Boothby and Sandiford (1920).

The body temperature (oral), the pulse rate, and both systolic and diastolic blood pressure were determined at each test. This was done toward the end of the rest period after the subject had been lying down at least fifteen minutes. Tests were run on each subject two or three times a week. During the last three months of the experiment four of the most reliable of the subjects were selected and tests were run on each of these daily except Sunday. During this period two male subjects were run daily, the procedure for them being the same as that already described for the women. A graph was prepared for each subject upon which the metabolic rate, pulse rate, body temperature, and blood pressure were all shown. A close inspection of these graphs usually indicated a direct correlation between pulse rate and metabolic rate, but this correlation was by no means perfect. No relationship between the metabolic rate and any of the other measurements made was apparent. A careful study of the correlations between these various physical measurements is being made and it is our intention to make it the subject of a future paper.

At the end of the experiment the tests made on each subject during menstrual periods were grouped together and the arithmetical mean computed. The standard deviation and the probable error of the mean were then determined. All tests that had been made between menstrual periods on the same subject were then placed in a second group and subjected to the same mathematical treatment. The difference between the arithmetical mean of the menstrual tests and the non-menstrual tests was then determined and the probable error of this difference was computed by taking the square root of the sum of the squares of the probable errors of the two means. The difference between the two means was then divided by the probable error of the difference and this number used as an index of the significance of the deviation between the average of the menstrual tests and that of the non-menstrual tests. These statistical data are shown in Table I.

It will be noted from this table that fourteen of the twenty subjects showed a lowering of the metabolic rate during the menstrual period. In one subject the average metabolic rate during the menstrual periods was exactly the same as the average rate between menstrual periods and the remaining five subjects showed a slight increase in the metabolic rate during menstruation. The subjects showing decreased metabolic rate during menstruation have been arranged according to the decreasing

TABLE I

COMPILATION OF STATISTICAL DATA SHOWING THE TENDENCY FOR THE BASAL METABOLIC RATE OF WOMEN TO BE LOWERED DURING THE MENSTRUAL PERIOD

Subject	Intermenstrual period			Menstrual period			Diff.	P.E. of diff.	Diff. divided by P.E.
	No. of tests	Avg. Cal. per sq. m. per hr.	Stand. dev.	No. of tests	Avg. Cal. per sq. m. per hr.	Stand. dev.			
A.C.	14	34.0	1.11	2	31.3	0.40	−2.7	0.28	9.7
M.P.	12	39.9	2.17	2	36.7	1.20	−3.2	0.71	4.5
L.S.	13	35.5	3.74	4	32.1	0.89	−3.4	0.83	4.1
S.R.	52	36.3	1.83	13	34.9	1.70	−1.4	0.36	3.9
R.D.	12	36.5	1.76	3	35.1	0.33	−1.4	0.37	3.7
F.R.	91	33.3	1.89	54	32.6	1.66	−0.7	0.20	3.5
D.W.	11	41.6	2.45	3	39.1	2.05	−2.5	0.94	2.7
A.B.	78	35.8	3.20	17	34.9	2.14	−0.9	0.43	2.1
E.C.	18	36.3	2.79	4	34.7	2.08	−1.6	0.83	1.93
C.B.	13	35.6	2.82	3	34.0	1.72	−1.6	0.85	1.88
N.R.	8	38.4	1.61	2	36.4	3.25	−2.0	1.58	1.26
M.K.	19	40.8	2.38	6	40.5	1.43	−0.3	0.54	0.55
I.K.	10	38.6	1.55	4	38.1	3.01	−0.5	1.06	0.47
R.P.	40	33.8	1.35	19	33.7	1.54	−0.1	0.28	0.36
B.P.	11	37.1	0.72	2	37.1	0.15	0.0	0.16	—[1]
R.S.	13	33.3	2.01	2	33.7	0.10	+0.4	0.38	1.05
G.J.	12	35.8	3.01	2	36.6	0.90	+0.8	0.73	1.11
M.B.	12	33.8	1.19	4	34.9	1.37	+1.1	0.87	1.26
M.S.	13	34.2	3.04	3	37.2	4.13	+3.0	1.71	1.75
M.C.	18	38.4	2.63	6	39.6	1.66	+1.2	0.62	1.94

value of the quotient obtained when the difference between the two means is divided by the probable error of this difference, while those showing an increased metabolism during menstruation have been arranged according to the increasing value of this quotient. At the top of the table comes A. C. Her metabolic rate during menstrual periods was 2.7 Calories per hour per square meter of body surface less than at other times. The probable error of this difference was only 0.28 Calories; therefore, the quotient

obtained when the difference is divided by its probable error is 9.7. According to the law of probability a difference as much greater than its probable error as this one is would occur fortuitously only once in several hundred billion cases. The second subject, M. P., had a metabolic rate during menstrual periods which averages 3.2 Calories less than her metabolism at other times. This difference is 4.5 times as great as its probable error. A deviation as much greater than its probable error as this would occur by chance only once in about 415 cases. Now it happens that the results obtained on these two subjects might be questioned, since in each case there were only two tests made during menstrual periods and in both cases these two tests were made during the same menstrual period. As previously stated, the tests on all subjects extended over at least two complete menstrual cycles. However, in the case of these two subjects the first test was made near the end of a menstrual period. As already explained, our regular procedure was to omit this first test. Since the experiment was stopped with these two subjects just before the beginning of the menstrual period at the end of the second cycle, this leaves only the one menstrual period for consideration in the final data. However, even if we should omit these two subjects the data would be only slightly less significant. Statistically, any deviation more than 1.8 times its probable error is usually considered significant. Ten of the twenty subjects show minus deviations occurring during the menstrual periods which are more than 1.8 times their probable errors. If we omit the first two, which might be questioned although we believe them to be perfectly valid, we find that according to the laws of chance, negative deviations in the metabolic rate during menstruation would occur fortuitously in the number and size noted only in some 450 cases instead of occurring, as they do in our data, in 18 cases. This means that these deviations are some twenty-five times more numerous than could be accounted for by chance.

On the other hand, of the five cases which show a positive deviation of the metabolic rate during menstruation, there is only one in which the deviation is more than 1.8 times its probable error. Positive deviations of this size and number would occur fortuitously in ten cases. It appears, therefore, that positive variations occur in our data only about half as frequently as could be accounted for on the basis of chance, while negative variations occur some 25 times more frequently than could be expected were they entirely fortuitous. If we include subject M. P., this number would be nearly doubled while the inclusion of A. C. would send it up into the millions. This seems to be excellent proof of the existence of a

tendency for the metabolic rate of women to be lowered during the menstrual period.

This lowering of the metabolic rate is of slight extent. The average for the ten subjects that showed statistically significant deviations is 5.3 per cent while if all fourteen subjects who show lowered metabolic rates during menstruation are included, the decrease amounts to 4.3 per cent. It would not be difficult to cover up a variation of this magnitude by some uncontrolled factor which acted in the opposite direction. For example Boothby and Sandiford (1924) have suggested pain and discomfort as the true cause of the apparent increase in the metabolic rate of women during menstruation which has been reported several times in the earlier work. It is now universally recognized that this factor must be guarded against. It is altogether probable that there are many other factors, as yet unrecognized, which might cause apparently fortuitous variations in the metabolic rate. The amount of food and exercise taken during the preceding twenty-four hours, the amount of sunlight that has reached the skin, the amount of sleep taken the preceding night or the mental state of the subject might very well produce noticeable changes in the metabolic rate. In this connection we have noted at this laboratory that students who have been at some social function the night preceding the test usually show a lowered metabolic rate. An effect of this same sort was observed during the course of the experiments on M. C. reported in this paper. This young woman was a student in the graduate school of the university. She was under observation for about three months and during this period she took the series of oral and written examinations that are required of all students before they are admitted to candidacy for the degree of Doctor of Philosophy. The average of all tests made on her before she had completed these examinations gave her a metabolic rate of 40.12 Calories per hour per square meter of body surface. After all of her scholastic work had been finished for the school year, her metabolic rate was determined on seven different days scattered over a period of more than four weeks. The average of all of these tests was 35.89 Calories per hour per square meter of body surface, a drop of 10.5 per cent from her previous level. We can suggest no cause for this sudden drop in the metabolic rate of this subject other than mental letdown which naturally accompanied the successful completion of her examinations. We have noted in a number of other cases that the metabolic rates of students were distinctly higher during the week of final examinations than at other times.

F. R. furnishes another example of an effect apparently due to the

mental condition of the subject. At the time these tests were made this subject was finishing her junior year in the College of Medicine. On the evening of June 1, 1928, she was on duty for the first time at the University Dispensary and returned to her room very much excited by this experience. The following morning, the third day of her menstrual period, she had a metabolic rate of 42.35 Calories per hour per square meter of body surface. The average of 54 tests run on this subject during menstrual periods was 32.60 Calories per hour per square meter of body surface, while on the morning of June 1, her metabolic rate had been 31.95 Calories and on June 4 it was again down to 33.23 Calories per hour per square meter of body surface. Here again we can offer no explanation for this very temporary increase in the metabolic rate other than the mental condition of the subject. It is our opinion that in many cases the rather slight change in metabolic rate which accompanies menstruation is concealed by such factors as those enumerated above.

Statistical treatment of our results has been limited to the testing of the significance of the change in the metabolic rate which occurs during the menstrual period itself. Inspection of the curves obtained when the results on individual subjects were plotted shows that this fall in the metabolic rate which accompanied the actual catamenia is not the only cyclic variation in the metabolism to manifest itself. In the great majority of cases the curves show a marked premenstrual rise similar to that emphasized by Rowe and Eakin (1921) and Wakeham (1923). The recently published data of Griffith *et al.* (1929) and of Benedict and Finn (1928) also indicate a premenstrual rise in the metabolic rate. Further discussion of this point, therefore, seems unnecessary. It is sufficient to state that during the week preceding the onset of the menstrual flow our subjects usually showed metabolic rates from five to ten per cent above the average value for the intermenstrual period.

In Figure 1, a portion of the results obtained upon three of the subjects are shown graphically. These curves have been selected because they show most clearly what we have come to believe is the typical cycle through which the metabolic rate of women passes. It can be seen from these curves that the lowered metabolic rate which accompanies the menstrual period often lasts for several days beyond the duration of the menstrual flow. There is then a progressive increase in the metabolic rate which is followed by a second drop which occurs at about the end of the second week of the cycle. This intermenstrual low point is of brief duration, usually lasting for but a day or two. Then comes the premenstrual rise and the drop in the metabolic rate which occurs with the onset of menstru-

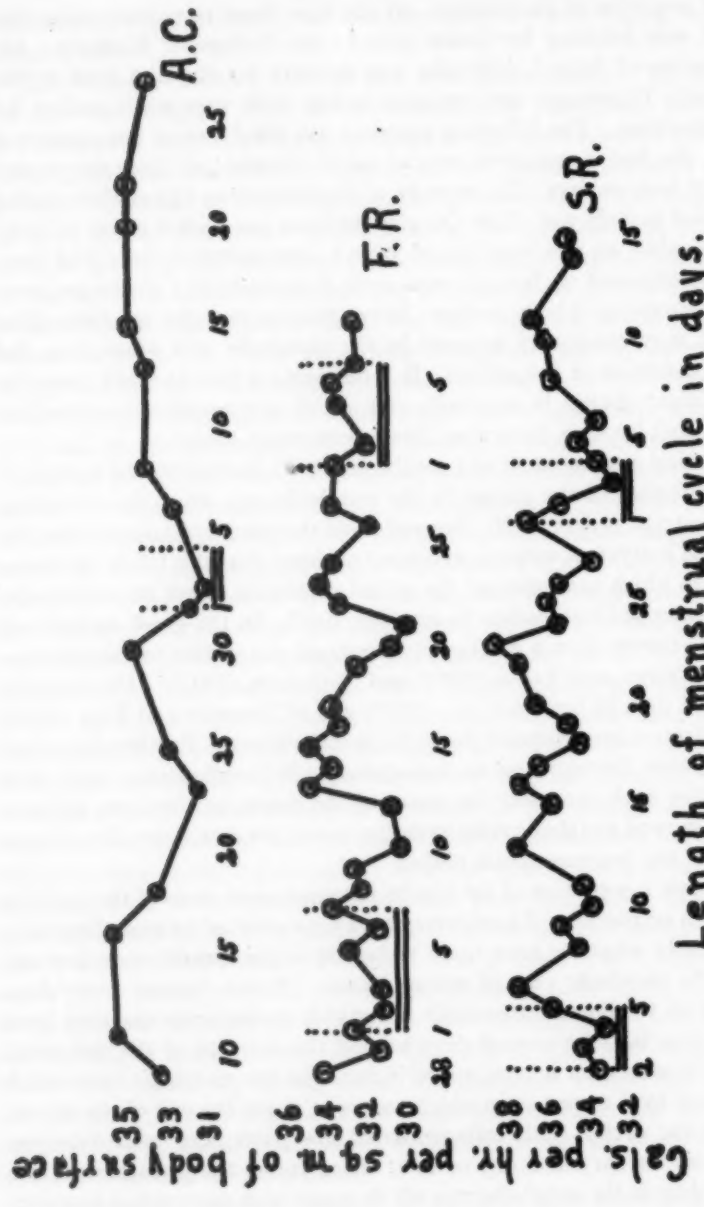

Figure 1. Graphic representation of a part of the results obtained on three subjects.

ation. It is probable that in some cases where this intermenstrual low point failed to appear, as for example in the second part of the graph for the subject A. C. shown in Figure 1, this was due to the fact that a test was not made on the particular day on which the metabolic rate was at this low level. With most of the subjects it must be remembered that tests were run only two or three times a week.

TABLE II

COMPILATION OF DATA SHOWING THE OCCURRENCE AND MAGNITUDE OF THE INTERMENSTRUAL DROP IN THE METABOLIC RATE

Subject	No. of menstrual cycles studied	Avg. length of cycle in days	No. of intermenstrual drops noted	Day on which intermenstrual drop occurred	Magnitude of intermenstrual drop in per cent	Magnitude of menstrual drop in per cent
A.C.	2	32	1	23	7.0	7.9
M.P.	2	30	1	15	3.8	8.0
L.S.	2	29	1	17	10.0	9.6
S.R.	5	28	5	12	3.1	3.9
R.D.	2	29	2	14	1.6	3.8
F.R.	9	28	5	13	6.8	2.1
D.W.	2	30	0	—	—	4.9
A.B.	9	30	6	13	2.8	2.5
E.C.	2	32	2	13	8.7	4.4
C.B.	2	29	2	19	6.5	4.5
Average	3.7	29.7	2.5	14	5.03	5.16

In Table II, the occurrence of these intermenstrual low points for the ten subjects that showed a significant lowering of the metabolic rate during menstruation has been tabulated. For these ten subjects there was a total of thirty-seven menstrual cycles studied and in twenty-five of these a low point occurred approximately midway between menstrual periods. These low points occurred in nine of the ten subjects included in the table, D. W. being the lone exception. There were only eleven tests made on this subject during the two intermenstrual periods she was under observation, the smallest number made on any subject in this group, and it is therefore altogether possible that the low points occurred on days when no test was made. Likewise, it seems probable that the other subjects would have shown these low points more frequently if the tests had been made at shorter intervals. As it is, they occur in about seventy per cent of the cycles. This is too large a percentage to be accounted for on the basis of chance. In extent they range from 1.6 to 10 per cent below

the average for the intermenstrual periods. The average for the group of ten including D. W. is 5.03 per cent. It is interesting to note that this is the same order of magnitude as the fall which occurs during the menstrual flow. For the same ten subjects the metabolism during menstruation averaged 5.16 per cent lower than during the intermenstrual periods. It is also noticeable from the table that there is a high degree of correlation between these two drops; that is, subjects who show the most pronounced drop in the metabolic rate during menstruation usually show the most marked intermenstrual low point.

The experiments of Benedict and Finn (1928) on Miss W. show similar intermenstrual low points. The thirty-three tests run on her during intermenstrual periods showed an average oxygen consumption of 179.8 cc. per minute. On February 29, the sixteenth day of the menstrual cycle, it dropped down to 176.0 cc. per minute and on March 28, the eighteenth day of the following cycle, it dropped to 175 cc. per minute. These two intermenstrual low points are 2.4 per cent below the average for the intermenstrual period as a whole, while the eleven tests made during menstrual periods average 3.3 per cent lower than the intermenstrual average. It is evident, therefore, that the results of Benedict and Finn as well as our own agree with the earlier work of Collett and Liljestrand (1924) in showing the existence of this intermenstrual low point in the metabolic rate.

It appears to be very well established that ovulation occurs 14 to 18 days after the beginning of menstruation, and since the average for this group of ten subjects shows that this drop in menstruation occurs about 15 days after the beginning of menstruation, while with Miss W. it occurred once on the sixteenth and once on the eighteenth, it seems probable that the two phenomena occur approximately simultaneously. The existence of a causal relationship between the two is a question upon which we can only speculate. There has been published recently some evidence which indicates that the metabolic rate in women may be influenced by the amount of ovarian hormone in the blood stream. McClendon, Burr, and Conklin (1929) in a preliminary report have noted the effect on the metabolic rate of women of injecting varying amounts of the ovarian hormone. In two of their five subjects the metabolic rate rose when small doses were injected and fell with the administration of larger doses. Two subjects showed increased metabolic rates following a single injection of a large dose of the hormone, while the fifth subject who received the largest dose of all showed an unchanged metabolic rate. It is possible that these results are complicated by the different

stages of the oestrus cycle in which the various subjects were at the time of the experiment. We shall await the complete report of this work with considerable interest. However, considering this evidence in connection with our own work, we are tempted to suggest that large amounts of the ovarian hormone in the blood stream cause a decrease, while smaller amounts produce an increase in the metabolic rate of women. It is not unreasonable to suppose that relatively large amounts of the ovarian hormone are set free in the blood stream at menstruation and at ovulation. This condition would then, according to our hypothesis, cause a lowered metabolic rate while the smaller amounts of the hormone present in the blood stream at other times would result in a higher metabolic rate.

The fact that two of our subjects were under observation for more than a year (in one case the summer months being omitted) and two others during the winter and spring, makes it possible to use our data in a consideration of the effects of the seasons on the basal metabolic rate. In Table III, the averages for the seasons during which these four subjects

TABLE III

DATA SHOWING THE EFFECT OF SEASONS ON THE BASAL METABOLIC RATE

Subject	Spring		Summer		Autumn		Winter		Spring	
	No. of tests	Avg. Cal. per sq. m. per hr.	No. of tests	Avg. Cal. per sq.m. per hr.	No. of tests	Avg. Cal. per sq. m. per hr.	No. of tests	Avg. Cal. per sq. m. per hr.	No. of tests	Avg. Cal. per sq. m. per hr.
F.R.	8	33.80	20	33.13	7	35.72	21	33.52	37	33.00
A.B.	12	37.53			14	36.86	16	34.84	36	35.52
S.R.							13	38.15	39	35.71
R.P.							10	33.03	30	34.16

were under observation are tabulated. In obtaining these figures all non-menstrual tests run during any particular season were averaged. It may be seen from this table that F. R. showed a metabolic rate of 33.80 Calories per hour per square meter of body surface during the spring of 1927. There was a slight decrease in this rate during the summer followed by a sharp rise in the autumn. During the subsequent winter and spring it dropped back to a point slightly lower than that noted at the beginning of the experiment. The outstanding characteristic of the records on this subject is the increased metabolic rate shown during the autumn. It is

interesting to note that during the fifteen months this subject was under observation her only vacation occurred in September 1927, and it is the tests run during the two or three months immediately following this vacation that show the increased metabolic rate. This seems to support the theory recently advanced by Benedict (1928) that the basal metabolism is to some extent a measure of the physical fitness of the individual. Subject A. B. was not available for test during the summer months. Her metabolism was only slightly lower in the fall than it had been during the preceding spring. During the winter, however, there was a marked drop followed by a rise in the following spring. The fact that this subject showed a lower metabolic rate in the spring of 1928 than she had in spring of 1927 again can be correlated with her physical condition. During the spring of 1928 she was in rather poor shape physically and from the 18th of April to the 8th of May was ill with what was diagnosed as influenza. Of the two subjects who were under observation during the winter and spring of 1928 S. R. showed a decrease while R. P. showed an increase in the metabolic rate. The same discrepancy in the point at which the metabolism starts up is seen in the case of F. R. and A. B., the former showing decrease and the latter an increase with the coming of spring. The two subjects whose metabolism was lower in the spring were older women who worked harder and probably took less time for recreation than the two subjects whose metabolism increased with the coming of spring. On the whole, our data seem to agree with those published by Gustafson and Benedict, (1928) who showed that the basal metabolism was usually higher in the summer than in the winter.

Summary

A study of the day-to-day variations in the basal metabolism of twenty women justifies the following conclusions:

1. There is a strong tendency for the metabolic rate to be lowered during the menstrual period.

2. A second low point in the metabolic rate occurs about the middle of the intermenstrual period.

3. In many cases the mental state of the subject seems to have a marked effect on the basal metabolic rate.

4. There seems to be a seasonal variation with the lowest metabolic rate occurring in the winter or spring and the highest in the summer or autumn. The time of year at which the metabolic rate begins to increase seems to depend to some extent on the personal habits of the subject.

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interesting to note that during the fifteen months this subject was under observation her only vacation occurred in September 1927, and it is the tests run during the two or three months immediately following this vacation that show the increased metabolic rate. This seems to support the theory recently advanced by Benedict (1928) that the basal metabolism is to some extent a measure of the physical fitness of the individual. Subject A. B. was not available for test during the summer months. Her metabolism was only slightly lower in the fall than it had been during the preceding spring. During the winter, however, there was a marked drop followed by a rise in the following spring. The fact that this subject showed a lower metabolic rate in the spring of 1928 than she had in spring of 1927 again can be correlated with her physical condition. During the spring of 1928 she was in rather poor shape physically and from the 18th of April to the 8th of May was ill with what was diagnosed as influenza. Of the two subjects who were under observation during the winter and spring of 1928 S. R. showed a decrease while R. P. showed an increase in the metabolic rate. The same discrepancy in the point at which the metabolism starts up is seen in the case of F. R. and A. B., the former showing decrease and the latter an increase with the coming of spring. The two subjects whose metabolism was lower in the spring were older women who worked harder and probably took less time for recreation than the two subjects whose metabolism increased with the coming of spring. On the whole, our data seem to agree with those published by Gustafson and Benedict, (1928) who showed that the basal metabolism was usually higher in the summer than in the winter.

Summary

A study of the day-to-day variations in the basal metabolism of twenty women justifies the following conclusions:

1. There is a strong tendency for the metabolic rate to be lowered during the menstrual period.

2. A second low point in the metabolic rate occurs about the middle of the intermenstrual period.

3. In many cases the mental state of the subject seems to have a marked effect on the basal metabolic rate.

4. There seems to be a seasonal variation with the lowest metabolic rate occurring in the winter or spring and the highest in the summer or autumn. The time of year at which the metabolic rate begins to increase seems to depend to some extent on the personal habits of the subject.

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